

PELARGONIUM LEAVES AS BIOACCUMULATOR OF POLYNUCLEAR AROMATIC HYDROCARBONS: ANALYSIS AND EVALUATION OF SOURCES AND AIR QUALITY IN PALERMO AREA

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SUMMARY

The leaves of *Pelargonium zonale* have been chosen because this plant is widespread in both the Mediterranean metropolitan and peripheral areas. The paper describes the analytical method, and the results concerning the determination of PAHs in *Pelargonium* leaves collected in the Palermo area, and their possible origins. The GC-MS method was applied, since identification of all the different compounds with spectra libraries is possible. Samples were analyzed for 22 PAHs. The reported analytical method is complete within 2 hours, and more convenient than that of other researches.

Total PAH concentrations in leaves varied between 24-357 µg/kg d.w. The relative abundance of the analyzed PAHs can be used to elucidate the sources. PAH concentrations were positively correlated to those of particulate measured by traditional methods.

The results clearly indicate that the chosen PAH levels in *Pelargonium* leaves offer the possibility to assess the quality of air in determined geographical areas. The city of Palermo is prone to a rather high level of atmospheric pollution, in particular in the zones with heavy traffic. Industrial activity is widespread in the area, but not an important source of the pollutants studied here.

KEYWORDS: Pelargonium, bioaccumulator, PAHs, origin, Palermo, GC/MS analysis.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are molecules of great interest because of their carcinogenic, terato-

genic and toxic properties. A case in point, the PAH benzo[a]pyrene is referred to as one of the most potent carcinogens known [1].

The presence and distribution of PAHs in the environment is largely a product of the incomplete combustion of organic materials (petroleum, oil, coal and wood). They are present in considerable quantities in fossil fuels, from which they are released by combustion. A very small percentage of PAHs in the environment can be linked to natural causes, such as volcanic activity and forest fires. Anthropogenic sources, such as vehicles, heating and power plants, industrial processes refuse and open burning, are considered to be the principal environmental sources [2].

Air is normally monitored by special automatic instruments that sample and analyze its various physical and chemical parameters. Consequently, the costs of and difficulties in managing the monitoring of large urban and industrial areas appear to be high. Therefore, in the majority of cases, traffic pollution monitoring is limited to few fixed monitoring stations, located in areas at risk. A practical problem in analyzing environmental contaminants (heavy metals, organic compounds, etc.) is that they often occur at low concentrations, near or below the detection limit. In air, concentrations vary widely over time, and the number of analyses needed for statistical processing is high. Interpreting trace contaminant concentrations in air, and predicting the threat they pose to human life under variable physical-chemical conditions, is very difficult.

The traditional analyses of air quality should be combined with biomonitoring to overcome their limitations.

In general, biomonitoring of pollutants relies on the fact that they induce some modifications in special bioin-

dicators. Organisms can be used as bioindicators or bioaccumulators. In the first case, the plant or animal has a high sensibility towards specific pollutants, showing clear and quantifiable symptoms, but in the second case, the chosen organism must be able to tolerate and accumulate its parts.

There are many advantages by using organisms. They essentially provide information on the average variation in time and space of the concentrations of contaminants in the considered area.

The advantages of animals and plants used to value air quality are reported in elsewhere [3, 4]. Leaves [5-7], and parts of many plants and lichens [8,9] have already been used to monitor air quality.

In general for PAHs, the more volatile 2-and 3-ring compounds exist primarily in the gas phase of the atmosphere, and will tend to be deposited to plants via dry gaseous and/or wet deposition. The less volatile 5-and 6-ring PAHs are more likely to be deposited on the plant surface bound to particles in wet and dry deposition. For compounds of intermediate vapor pressure (4-ring PAHs, for example), a temperature-dependent gas/particle partitioning of PAHs will occur, such as that they are subject to both wet and dry deposition in gaseous and particle-bound form [10]. Physiological features of leaves play an important role in determining the scavenging efficiency and retention of air-borne particles on the leaves. Leaf surfaces vary greatly between plant species, both in morphology and chemistry of the cuticle, the number and distribution of stomata, and the presence or absence of hairs. The water repellence of leaves is important in determining how effectively the leaves can loose the particulate on their surface. With the cuticle on the upper leaf covered by branched pluri-cellular hairs, the leaves of the *Pelargonium* with their chemical nature are well-equipped to accumulate PAHs.

The purpose of this work is to present the method to analyse Palermo air quality, using leaves of *Pelargonium zonale* as bioaccumulator of PAHs.

The leaves of *Pelargonium zonale* have been chosen, because this plant is widespread in the Mediterranean area like other arboreal, shrub and herbaceous examples, and commonly found both in the metropolitan and peripheral areas of the city of Palermo.

Identification and quantification of PAHs in environmental samples is often complicated by a number of other compounds present in the sample. To avoid these problems, the GC-MS method was applied, since here it is possible to identify the different compounds with the aid of spectra libraries. Samplers were analyzed for 22 PAHs, sixteen of which have been listed as priority pollutants by the World Health Organization (WHO), the European Economic Community (EEC), and the U.S. Environmental Protection Agency (USEPA) [11], and 6 were chosen by us. This method has been applied to compare local concentrations of selected PAHs from 31 stations in some areas of Palermo.

This paper describes the analytical method and the most significant results concerning the chemical determination of PAHs in *Pelargonium* leaves, prevalently collected in the Palermo area, and also their possible origins.

MATERIAL AND METHODS

Chemicals and reagents. All the solvents were of HPLC grade, and water was purified by a Milli-Q system. The compounds used as internal standard were per-deuterated PAHs (Supelco, Milano). The standard solution containing 16 compounds was the so-called calibration mix B 31455 (Supelco 610). The standards of perylene and some methyl derivatives were prepared by us from Fluka chemicals.

Sampling. Chemical analyses were carried out on *Pelargonium zonale* leaves collected in the period autumn-winter 2001. The sites, which were chosen for the analysis of the leaves, are indicated in Table 1. The repeatability of sampling was preliminarily checked by analysing PAHs in 5 different samples of leaves collected at the same station. The standard deviations of sampling and analytical process were negligible (about 5%). For the successive analysis, from each station we collected about 10 g of leaves from two plants, and homogenized them before extraction.

29 sites are situated in different areas affected by various types of emissions, and two in the countryside around Cefalù, far from any possible source of PAHs.

50-100 g of leave samples were collected from different parts of the plants. Samples were collected using rubber gloves, separated from impurities, sealed into containers, immediately refrigerated (4 °C) to avoid exposure to light, and then rapidly carried to the laboratory where they were deep-frozen before analysis.

Determination of dry weight. About 2 g of homogenized sample was dried at 105 °C for one night. The water content was determined by weight loss, and utilized to correlate all the results with dry weights.

Extraction of PAHs from the leaves. Together with the PAHs analysis, recovery tests were carried out by using leaves not containing PAHs. After complete PAH extractions were obtained (by GC-MS analysis), a known amount of PAHs standard mixture (EPA) was added to each purified "blank" leave sample. Average recovery was calculated to be 70% for all the components.

2-g aliquots of leaves were extracted twice in an ultrasonic bath for 20 min with 20 ml of dichloromethane. The extracts, after having been filtered on Na₂SO₄ anhydrous, were concentrated by evaporating them at 35 °C, and drying them under a weak current of nitrogen. The dry residue was then dissolved in 0.25 ml of a cyclohexane solution containing per-deuterated internal standards and analysed by GC-MS.

TABLE 1 - Sites chosen for the analysis.

Denominations	Characteristics of the stations
Massimo Theatre	Pedestrian area
Teatro Politeama	Ring road frequented by very heavy and slow traffic
Piazza Croci	Route frequented by very heavy and slow traffic
Via Roma	Road frequented by very heavy traffic
Court	Route frequented by heavy and slow traffic
Via Dante	Road frequented by very heavy traffic
(Cathedral	Route frequented by very heavy traffic
Piazza A. De Gasperi	Large area frequented by very heavy and slow traffic
Viale Strasburgo	Large route frequented by heavy and slow traffic, the sample was taken far from the road
Via Notarbartolo	Area frequented by heavy traffic
Corso Calatafimi	Large area frequented by heavy traffic near a petrol pump
Viale Regione Siciliana	Large route frequented by very heavy traffic
Piazza Vittorio Veneto	Large area frequented by light traffic
Piazza Scaffa	Ring road frequented by heavy traffic
Industrial Area	Interested by industrial activities and frequented by light traffic
(University)	Inside a garden 300 m away from nearest trafficked urban area and protected by buildings
Via Paruta	Road frequented by light traffic and near a petrol pump
Via dei Cantieri	Road frequented by light traffic and near shipyard
Via Belgio	Route frequented by heavy traffic, the sample was taken 200 m away from the road
Viale Lazio	Located in a busy road interested by light traffic
Viale Michelangelo	Road frequented by light traffic
Via Oreto	Route frequented by heavy traffic
Via Ugo La Malfa	Industrial activity
Via Gustavo Roccella	Road frequented by light traffic
Via Ernesto Basile	Route frequented by heavy traffic
Botanical Garden	Inside a green area 200 m away from traffic and 200-300 m away from a plant for gas production (inactive from a pair of years)
Via Perpignano	Road frequented by light traffic, recently was stricken by a large scale wood fire
Partanna Mondello	Frequented by light traffic
Sferracavallo	Road frequented by light traffic
Barreca (Cefalù)	Distant from traffic and interested by agricultural practise
Capo Playa (Cefalù)	Distant from traffic, near the sea

TABLE 2 - List of groups of PAHs formed for analysis, the deuterated standards employed (underlined>, and the quantification and confirmation ions for SIM GC-MS mode.

Group	Chemical	Quantification ion	Confirmation ions
1	naphthalene (N)	128	64, 127
	2-methyl naphthalene (2mN)	142	115, 70
	1-methyl naphthalene (1mN)	142	115, 70
	acenaphthylene (Apl)	152	76, 151
	acenaphthene (Apt)	154	152, 76
	fluorene (F)	166	164, 165
	acenaphthene d ₁₀	164	
2	phenanthrene (P)	178	188, 89
	anthracene (A)	178	188, 89
	2-methyl anthracene (2mA)	192	96, 82
	9-methyl anthracene (9mA)	192	96, 82
	fluoranthene (Fl)	202	101, 200
	pyrene (Py)	202	101, 200
	1-methyl pyrene (1mPy)	216	108, 94
	benzo(a)anthracene (BA)	228	114, 226
	phenanthrene d ₁₀	188	
3	chrysene (Cy)	228	114, 226
	benzo(b)fluoranthene (BbF)	252	126, 250
	benzo(k)fluoranthene (BkF)	252	126, 250
	benzo(a)pyrene (BaPy)	252	126, 250
	chrysene d ₁₂	240	
4	perylene (Pr)	252	126, 250
	indeno(1,2,3-cd)pyrene (I)	276	277, 138
	dibenzo(a,h)anthracene (D)	278	279, 139
	benzo(g,h,i)perylene (B)	276	277, 138
	perylene d ₁₂	264	

PAH Analysis. The qualitative and quantitative determinations were carried out with a GC/MS combination (Shimadzu mod. GC-17A coupled to a Shimadzu quadrupole detector mod. GCMS-QP5000), operating with acquisition data (Shimadzu, CLASS 5000 system). The instrument is equipped with a split/split-less injector. Samples were injected split-less (2 min, total flow 20.6 ml/min. The injector temperature was maintained at 280 °C. The GC temperature program was: from 60 °C (3 min) to 295 °C (13 min) at 5 °C/min. The carrier gas was helium (flow rate 1.6 ml/min). The capillary column used was PTE-5, 30 m x 0.25 mm ID, and 0.25 µm film thickness (Supelco, Milano). The interface temperature was 300 °C. The analysis was operated in Selected Ion Monitoring (SIM) mode. The selection ions are reported in Table 2.

The identification of the standard mixture components was carried out by comparing retention times of each mixture component with those of pure components, analysed under the same experimental conditions. Identification was confirmed by comparing the spectra of the single components with those stored in the library of the acquisition system. The identification of PAHs in the solutions originating from the leaf extract was carried out on the basis of previously determined retention times, and confirmed by using mass spectra. In particular, we use two masses to

identify the analytes and one mass (molecular ion) for the deuterated internal standard compounds, because they are easily identified with higher concentrations (consequently areas) than the other components of the extracted solution. The sample PAHs were quantified, with regard to perdeuterated PAHs added to the dry residue. The response factors of different compounds were determined injecting a series of 22-component standards with concentration of perdeuterated PAHs identical to those used for spiking the samples. Alongside the analysis of unknown samples, recovery tests were carried out.

RESULTS AND DISCUSSION

The individual and total PAH concentrations (µg/kg leaf dry weight, averages of three replicates) for each of the 31 stations are shown in Table 3 and Fig. 1. Results varied from 24 µg/kg at station Viale Strasburgo (n° 9), located far from the road, to 357 µg/kg in Croci Square (n° 3), where the traffic is severe and slow. Phenanthrene, fluoranthene and pyrene are the three most abundant components in nearly all the samples.

Treatment of the data with Surfer 7 (Fig. 2), shows the spatial distribution of total PAHs in Palermo area.

TABLE 3 - Individual and total concentrations (the averages of two samplings) of PAHs (µg/kg d.w.).

N	2mN	1mN	Apl	Apt	F	P	A	2mA	9mA	Fl	Py	1mPy	BA	Cy	BbF	BkF	BaPy	Pr	I
0.03	0.05	0.05	0.02	0.02	0.03	0.08	0.05	0.02	0.02	0.04	0.04	<0.02	0.03	0.00	0.02	0.02	0.01	0.01	0.03
22.18	30.82	2.53	4.91	0.37	1.37	32.77	1.92	15.74	<0.02	21.70	29.16	2.51	3.74	13.30	8.73	8.23	3.47	0.17	1.66
3.64	1.65	3.16	0.51	0.65	<0.03	5.89	0.76	20.04	<0.02	3.94	5.91	0.14	0.10	0.99	0.23	0.21	0.22	0.46	0.23
6.46	3.51	1.10	0.90	0.24	0.29	7.09	0.99	2.99	<0.02	7.82	6.09	1.04	0.39	0.84	0.21	1.95	0.92	<0.01	0.38
4.57	0.28	0.84	0.53	0.21	1.75	5.28	0.81	22.72	<0.02	4.00	4.20	0.07	0.10	11.23	0.52	0.16	1.53	<0.01	3.56
16.04	3.04	3.04	6.73	2.10	5.64	35.31	5.08	10.19	4.38	34.34	56.8	2.09	0.90	34.77	44.86	6.34	16.54	1.37	14.56
17.28	5.91	4.17	1.63	0.52	36.78	16.42	0.95	28.84	<0.02	8.04	11.77	1.27	1.29	6.47	0.42	1.30	1.85	3.39	1.52
0.95	0.32	0.72	0.38	0.00	<0.03	1.38	0.50	5.79	<0.02	0.86	1.37	<0.02	0.10	0.00	0.00	1.58	0.28	<0.01	0.00
1.00	0.59	0.21	0.26	0.13	0.85	2.48	0.46	2.73	9.68	1.25	1.46	0.02	1.09	2.71	0.59	0.27	2.15	21.33	1.39
7.90	3.81	2.58	1.93	0.87	1.74	8.54	0.57	16.21	<0.02	5.02	6.39	0.19	1.88	4.39	2.46	<0.02	0.20	3.80	4.66
4.32	1.55	2.92	0.74	<0.02	1.40	5.30	0.50	7.69	21.27	2.56	3.63	0.64	0.10	1.53	0.95	2.20	0.00	2.32	0.00
2.26	0.73	0.11	0.21	0.38	2.62	2.26	0.92	2.70	5.72	1.28	2.29	<0.02	0.10	0.83	0.00	0.17	0.61	<0.01	0.00
< 0.03	0.38	0.19	0.67	<0.02	0.42	2.59	0.87	2.73	19.00	1.57	0.98	<0.02	1.61	4.84	0.00	0.00	0.08	26.74	8.74
5.86	1.31	0.90	3.53	0.28	3.15	20.92	1.95	8.56	<0.02	12.08	15.86	0.40	0.29	10.73	2.95	2.24	4.44	2.45	8.01
2.12	0.14	0.76	1.15	2.27	0.83	3.84	0.50	2.75	13.73	2.92	3.33	0.09	0.79	1.98	3.10	0.80	0.00	0.00	0.34
8.04	2.67	2.48	1.29	0.25	2.00	8.45	0.50	4.15	14.91	9.00	13.74	0.74	0.10	9.69	13.33	1.80	5.99	0.85	0.69
2.20	1.93	0.74	0.58	0.33	2.28	8.54	1.17	6.48	6.88	2.88	3.18	0.15	1.64	3.61	0.60	6.28	0.00	<0.01	1.86
5.41	1.54	2.05	1.12	0.57	1.59	6.85	0.50	8.12	12.56	2.32	2.30	0.14	0.10	1.47	1.26	8.24	1.66	<0.01	0.51
1.91	0.46	0.94	0.49	0.21	<0.03	4.51	0.53	5.09	<0.02	4.02	4.62	0.31	0.10	4.20	6.20	14.75	1.08	0.03	0.00
4.34	3.27	2.43	3.64	0.61	1.62	8.41	1.01	15.42	<0.02	7.15	7.31	0.38	1.99	5.97	8.42	<0.02	1.26	1.78	0.19
2.33	0.74	1.33	5.97	0.26	1.56	2.75	0.19	3.42	2.12	1.59	2.04	0.05	3.84	8.97	4.46	2.84	0.04	6.16	4.02
3.37	11.38	5.86	0.08	3.05	8.69	7.04	0.74	14.64	<0.02	4.92	4.06	<0.02	0.10	0.25	1.00	13.86	0.89	0.02	2.85
3.20	1.61	0.55	0.36	1.28	1.16	2.04	0.50	2.07	2.19	2.25	3.14	0.17	0.15	0.97	6.53	0.75	0.59	2.23	0.97
12.29	6.11	4.48	5.20	0.44	1.91	14.69	0.63	13.03	<0.02	13.74	15.01	0.03	2.80	14.00	1.98	24.53	6.88	<0.01	4.55
0.71	1.51	0.50	0.42	0.19	0.33	2.20	0.87	3.15	<0.02	1.58	1.71	0.03	2.62	6.56	0.29	10.04	0.62	61.69	1.21
1.32	0.84	0.61	0.27	0.29	1.89	1.52	0.50	2.46	<0.02	1.29	2.22	0.06	1.57	5.13	1.48	2.64	0.83	0.00	0.25
1.43	0.72	0.31	0.54	0.09	0.51	4.86	0.51	0.19	0.47	5.50	7.79	0.36	2.13	6.10	7.25	1.21	2.49	<0.01	0.58
30.79	1.73	2.98	1.60	0.09	0.91	5.58	0.50	15.92	<0.02	4.40	3.84	0.05	0.13	0.49	0.49	0.00	<0.01	9.33	0.21
22.13	1.39	2.36	1.17	0.91	0.76	3.71	0.50	1.64	<0.02	2.33	3.04	0.22	0.14	0.65	<0.02	<0.02	<0.01	2.08	<0.03
9.88	9.52	3.45	2.61	0.57	<0.03	18.50	1.47	13.09	<0.02	13.47	13.24	0.45	4.71	12.45	0.51	7.85	0.20	9.90	0.45
3.62	1.86	2.92	1.12	0.22	4.88	10.49	0.50	11.89	<0.02	2.48	1.67	0.25	1.07	2.80	<0.02	<0.02	0.24	<0.01	0.10
22.10	2.27	2.97	2.66	0.16	2.27	15.66	1.34	4.60	<0.02	12.64	16.54	0.45	2.19	3.81	0.36	0.58	2.16	3.01	0.10

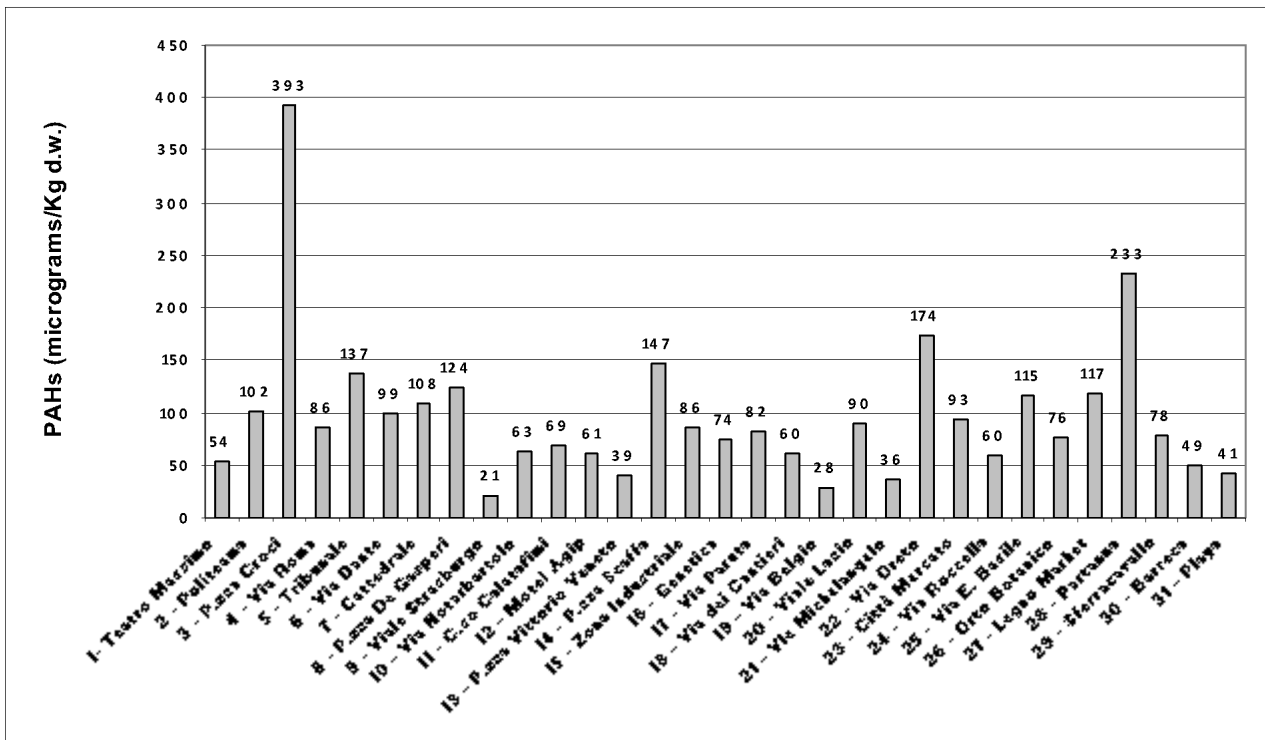


FIGURE 1 - Total distribution of PAHs in the samples.

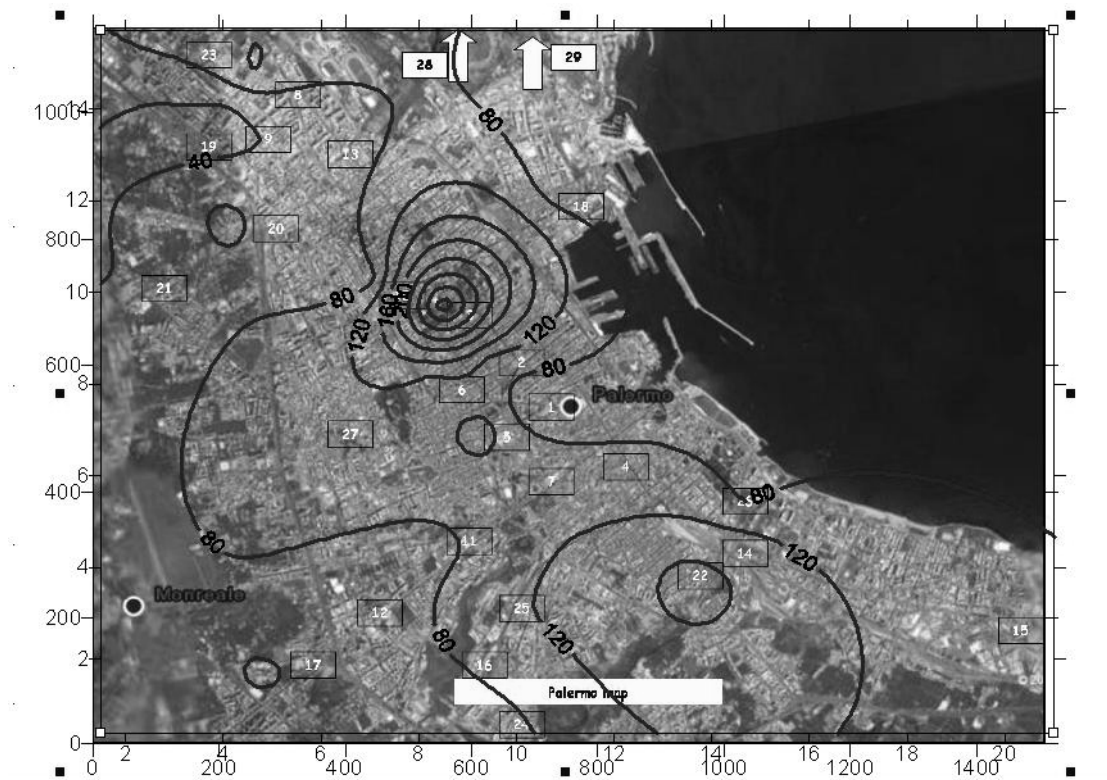


FIGURE 2 - Spatial distribution of total PAHs.

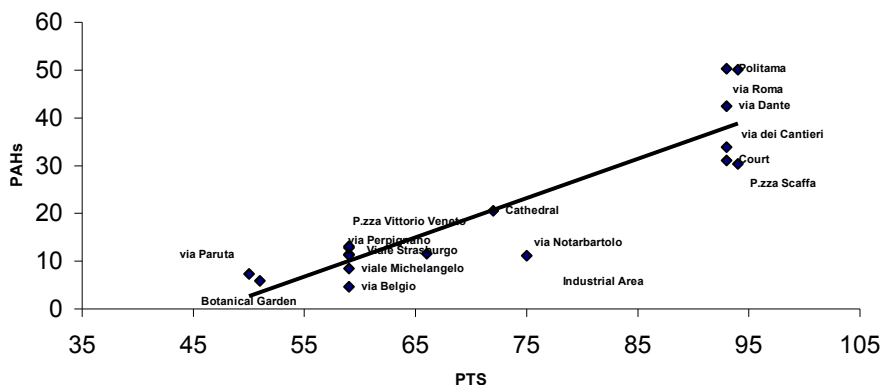


FIGURE 3 - Correlation between total PAH and particulate concentrations.

The greater concentrations of PAHs are to be found in the proximity of the historical town centre. This area is characterized by narrow streets frequented by heavy traffic consisting of cars and busses.

The compounds were grouped into classes according to the number of aromatic rings in their structures. 3-and 4-ring PAHs contribute more than 50 % to total PAHs. The total PAH burden at control sites (Barreca, Playa) was dominated by the low-molecular weight PAHs, while along the urban roads fluoranthene, pyrene and benzo[a]anthracene showed the highest values.

Figure 3 shows the correlation between total concentration of less volatile PAHs and that of particulates, gravi-

metrically determined on a filter (0.45 µm) by AMIA [12], aspirating a known volume of air at the various stations. For comparison, we used data from stations in which the traffic conditions and geographical position can be considered to be equivalent to those of sampling (Fig. 3).

The relative abundance of PAHs present can be used to elucidate origins and provide “fingerprint” sources [13, 14]. Phenanthrene is always much more abundant than the isomeric anthracene. In particular, phenanthrene is more thermodynamically stable than anthracene, so, in PAH petrogenic pollution the Ph/An ratio is very high, while during combustion processes the high temperatures favour anthracene formation lowering the ratio.

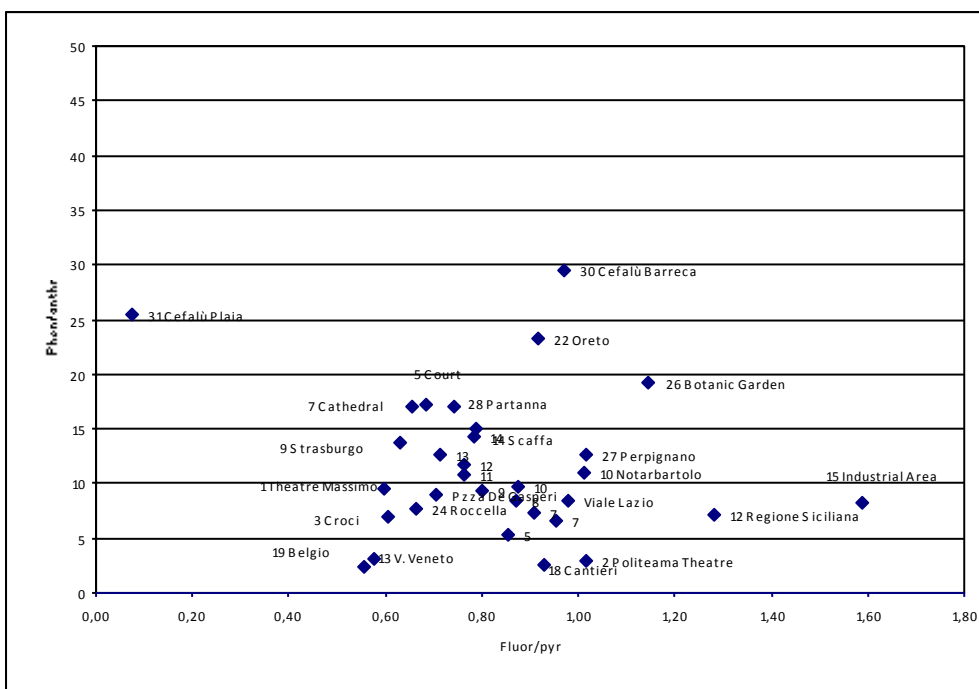


FIGURE 4 - Relationship between isomer PAH compounds.

With the aim to add a further contribution to the knowledge of the origin and environmental circulation of PAHs, we used Fl/Py and Ph/An distribution indexes in leaves.

The Fl-Py ratio can be applied to this purpose, and generally a ratio <1 indicating vehicular emissions, a ratio of ≈ 1.0 indicating that the contamination by PAHs was due to combustion processes (wood smoke) as source, and a ratio >1 ($\approx 1,4$) are characteristically for coal combustion emission [15]. Our data indicate that PAHs in the leaves collected in the major number of stations seem to be of vehicular origin (Fig. 4). While the station n° 15 (Industrial area) seemed to be interested by wood and coal combustion because of emissions from a cast iron foundry which uses fossil coal, and from a nearby coffee-making plant using wood to roast the coffee. The station n°26 (Orto Botanico) diverges from the others because the PAHs are emitted during city gas production. The stations that are outside of this range are compatible with emission of various natures.

CONCLUSIONS

The analytical method used is mostly convenient with respect to those utilized in other researches [6, 7]. Analysis is complete within 2 hours, compared to 12 hours necessary for the method employed before.

To evaluate the precision of the analysis, three replicates of same field samples were analyzed. The relative standard deviation (RSD) of the replicates of individual compounds ranged from 8 to 20%.

The detection limit (LOD), estimated as 3σ (three times the background noise) IUPAC criterion, was similar for all analyzed compounds (less than $0.01 \mu\text{g}/\text{kg}$ d.w. for all analytes). The blank values were reported in Table 3.

The data clearly indicate that the chosen PAHs determined in the leaves of *Pelargonium* offer the possibility to assess the quality of air in a determined geographical area.

In such a way, the need for long periods of sampling with complex and difficult-to-handle instruments and numerous analytical calculations are avoided. Moreover, these results provide mean information from the time of budding until final collection, not influenced, as occurs with traditional analyses, by meteorological conditions at the time of sampling.

The results of these investigations allow us to affirm that the *Pelargonium* is an excellent biological sampler, which can be easily implemented in research in Sicily, since it is characteristically of the area.

This type of survey is more rapid and economical than the traditional methods, and may, therefore, be used by small communities.

The results using *Pelargonium* as bioindicator have shown that the city of Palermo is prone to a rather high level of atmospheric pollution, in particular in the zones with heavy traffic. Industrial activity is wide-spread in the area, but not an important source of the pollutants studied here.

Also stored samples of leaves can be used for retrospective PAHs analysis, if the set was sampled over a long period.

A comparison with other studies of total PAH concentrations in Palermo area suggests that the levels are within the concentration ranges already reported [5-7].

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