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SUMMARY

The concentrations of 27 polycyclic aromatic hydro-carbons (PAHs), including six carcinogenic PAHs, were measured in leaves of *Quercus ilex* L. and surrounding soils in order to evaluate their suitability as biomonitors of PAH air contamination. Healthy and mature leaves and surface soils (0-5 cm) were collected at five urban sites and one control site in May and September 2001 and January and May 2002. The PAHs were extracted by sonication and quantified by GC-MS. Both leaves and soils showed PAH accumulations at the urban sites and spatial trends of PAH concentrations among the sites, although no correspondence was highlighted between the spatial trends among leaves and soil PAH concentrations. The temporal variations in PAH concentrations were wide for the leaves with the highest values in January, whilst for the soils they were narrow. The 3-ring PAHs were the most abundant in the leaves whilst the 6+7-ring PAHs were the most abundant in the soils. The proportions of the carcinogenic PAHs to the total were on average about 15% and 25% in the leaves and soils, respectively.

KEYWORDS:
PAH pollution, surface soil, carcinogenic PAHs, spatial and temporal trends, urban and control sites, *Quercus ilex* L.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants. In the atmosphere the more volatile 2- and 3-ring compounds exist in vapour phase, the less volatile 5- and 6-ring ones bound to the particles, and the intermediate volatile 4-ring compounds both in vapour and particle-bound forms. The partitioning in vapour or particle phase depends on the air temperature and the chemical-physical characteristics of each PAH [1-3]. PAHs are widespread in the air of natural, rural, urban and industrial areas [4-7], because the emission sources are both natural and anthropogenic [8]. The former include volcanic emissions and fires, the latter include industrial activities, incineration waste and vehicular traffic. Besides, PAHs can be transported far from the emission sources reaching places where emissions are scarce [9]. Air PAHs accumulate on soils and canopies by wet and dry depositions. The accumulation of PAHs on canopies depends on leaf morphology. Leaves with hairs and thick layers of lipids and waxes favour the absorption of PAHs on leaf surfaces [10-13]. Soils without canopy cover reflect the air PAH depositions, whereas soils below can-opies also reflect the effect of the stem-flow and the washing of the leaves due to rain [14]. A fundamental role on PAH soil concentrations is also played by the presence of litter or grass [15] and microbial decomposition [16].

This work aimed to compare PAH concentrations in holm oak leaves and surrounding soils in the urban area of Naples (southern Italy) in order to evaluate their capacity to accumulate PAHs. Detection of pollutant accumulation in natural matrices, due to air deposition, allows us to define PAH contamination gradients. Therefore, soils and leaves could be useful low-cost monitors of air quality to extend the number of measuring points, thus providing a support for instrumental monitoring.

MATERIALS AND METHODS

The study area covered five urban sites (VA, PT, TG, VM and VC) located in the city centre of Naples. A natural holm oak wood in the Vesuvius National Park (VS), far from urban centres, was used as control site. Naples is a very densely populated city, characterised by a high traffic load. At all the urban sites the samplings were carried out in public gardens and large flower beds. The soils at VA and PT are compact and have little grass cover and scant litter; by contrast, at TG and VM they are

soft and show extensive grass cover; at VC the soils are mostly covered by lawns. Sites VA and PT are affected by a very high traffic load; VM is also affected by intense traffic load despite being situated in public woodland. Site TG is along the motorway and VC is close to the seaside in a very open windswept area.

Mature and healthy leaves of *Quercus ilex* L. were collected in May and September 2001 and January and May 2002 from at least ten specimens. Simultaneously, after removing the litter, surface soils (0-5 cm) under the canopy of the same specimens were sampled. All leaf samples were stored at –20 °C in polyethylene bags until analysis, while all soil samples were sieved (mesh size 2 mm) and air-dried. All analyses were carried out on 3 sub-samples.

The extraction procedure was carried out on defrosted leaf and air-dried soil samples. All samples (ca. 5 g wet weight and ca. 30 g dry weight, respectively) were mixed with equal quantities of anhydrous sodium sulphate (i.e. ca. 5 g and 30 g for leaves and soils, respectively). A mix of deuterated PAHs (naphthalene D8, acenaphthene D10, phenanthrene D10, chrysene D10 and perylene D12) were added before extraction for quantification. The samples were extracted in a mixture of dichloromethane:acetone (1:1, v:v) by 3 consecutive sonications (Misonix, XL2020). After the first sonication (3 min) in 100 ml of the mixture, the samples were recovered by vacuum filtration and the extracts stored in a flask. The recovered samples were sonicated (3 min) again, adding 100 ml of mixture, and consecutively a third sonication (3 min) was carried out adding another 100 ml of the mixture. After the third sonication, the samples were vacuum-filtered and the extracts added to that of the first sonication. The combined extracts were rotary-evaporated to ca. 5 ml, filtered (0.2 μm), and gently dried under a nitrogen stream. Subsequently, they were diluted with 4 ml of dichloromethane to be analysed.

The analyses were carried out by GC-MS (Hewlett Packard 5890 GC with on-column manual injection, coupled to Hewlett Packard 5971 mass-selective detector, and equipped with an HP-5MS capillary column, 30 m x 0.25 mm i.d. x 0.25 μm film thickness and a phase ratio of 250). Oven temperature program was held at 33 °C for 1 min, then ramped at 20 °C/min to 280 °C and held for 15 min. The on-column inlet temperature was set in the oven track, with an injector temperature 3 °C higher than the oven temperature at all times. The SIM (selected ion monitoring) modality of acquisition was used. The carrier gas was helium at a constant flow rate of 1 ml/min during the analysis. The calibration curve for each PAH was carried out.

Twenty-seven PAHs were quantified (naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, 2,3-benzofluorene, benzo(c)phenanthrene, benzo(g,h,i)fluoranthene, benzo(b) naphtha(1,2-d)thiophene, cyclopenta(c,d)pyrene, benzo(a) anthracene*, chrysene + triphenylene, benzo(b*+k*+j) fluoranthene, benzo(e)pyrene, benzo(a)pyrene*, 1,3,5-tri-phenyilbenzene, perylene, indeno(1,2,3-c,d)pyrene*, benzo(b)chrysene + picene, dibenzo(a,c)anthracene, dibenzo(a,h)anthracene*, benzo(g,h,i)perylene, anthanthrene, and coronene). The sum of the 27 compounds listed above constitutes ΣPAHs; the asterisks indicate carcinogenic PAHs [17]. The deuterated PAHs were also quantified and percentage recovery was included in the calculations to quantify all the investigated PAHs.

Moreover, the soil organic matter contents were also detected in order to evaluate the relationships between PAH soil accumulation and organic matter. It was measured via loss on ignition at 550 °C for 2 hours.

The data were processed by statistical tests; the normality of the distribution of the data sets was assessed by the Kolmogorov-Smirnov test. The significance of the differences were performed by ANOVA test followed by post hoc test (Student-Newman-Keuls test), and the correlations by Pearson's test. The coefficients of variation (percentages of the ratios between standard deviation and mean values) were calculated in order to evaluate the variations in the PAH concentrations among the urban sites both for the leaves and soils.

RESULTS

ΣPAHs leaf and soil concentrations at each urban site were higher than at the control site for all the samplings (Fig. 1), and among the urban sites spatial trends were observed both for leaf and soil ΣPAH concentrations (Fig. 1). Over the whole investigated period, at the urban sites, leaf ΣPAH concentrations were 2- to 9-fold and soil ΣPAH concentrations 2- to 27-fold higher than that at the control site (Fig. 1). Among the samplings, the leaves at all the sites showed differences in ΣPAHs with significantly higher concentrations (at least $P<0.05$) in January (Fig. 1). By contrast, the soils showed no differences. Only at VA they were particularly high in May 2001 and 2002. Soil ΣPAH concentrations were correlated ($P<0.01$) negatively to soil organic matter contents (Fig. 1, Table 1).

TABLE 1
Mean values (± standard error) of soil organic matter contents
(% d.w.) at the control and urban sites for all the samplings.

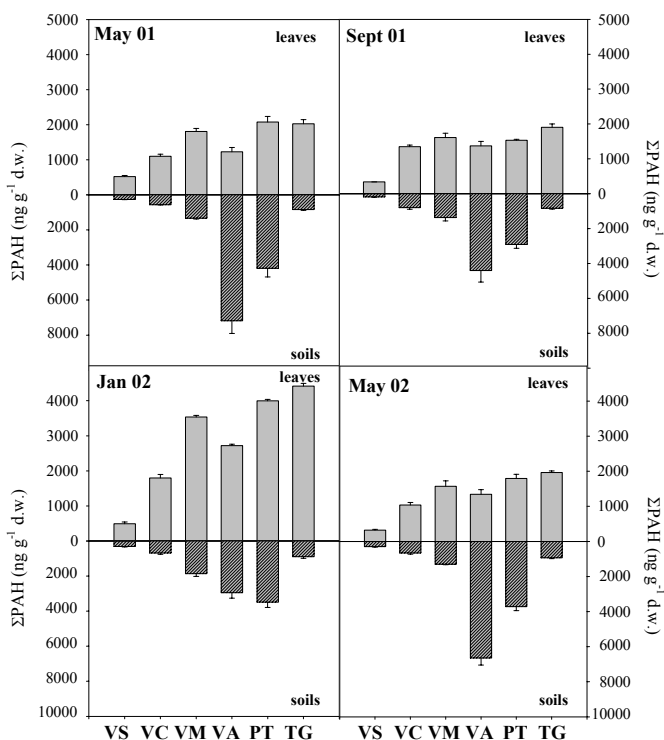| Soil organic matter contents	VS	VC	VM	VA	PT	TG
Mean values	28.77	9.18	26.19	8.06	7.63	16.15
Standard error	1.05	0.79	1.22	0.28	0.72	0.64

FIGURE 1
Mean concentrations (± standard error) of ΣPAHs in *Q. ilex* leaves and surface soils at
the control and urban sites in May 2001, September 2001, January 2002, and May 2002.

Among the urban sites, variations in ΣPAHs were observed both for leaves and soils at each sampling time. The coefficients of spatial variation in ΣPAH concentrations for the leaves were narrower than for the soils (Fig. 2). Indeed, for the leaves the calculated coefficients ranged from
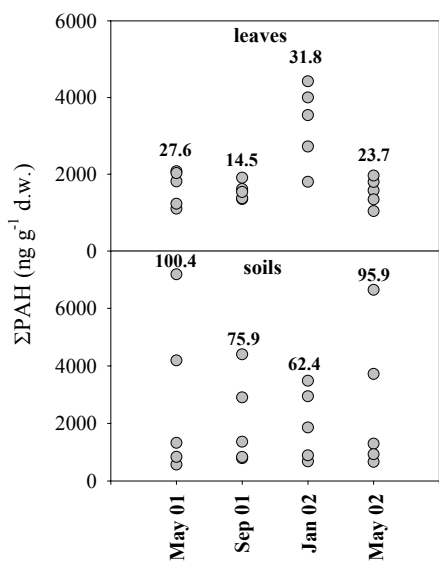
about 15% to 32% (Fig. 2) and for the soils from about 62% to 100% (Fig. 2). For the leaves, the highest coefficient of spatial variation in ΣPAH concentrations was calculated in January, when the lowest coefficient of variation was calculated for the soils (Fig. 2).

The leaf/soil ratios of ΣPAHs were always higher than 1 at the control site (Fig. 3), whilst they ranged from 0.2 to 4.9 at the urban sites depending on sites and sampling time (Fig. 3).
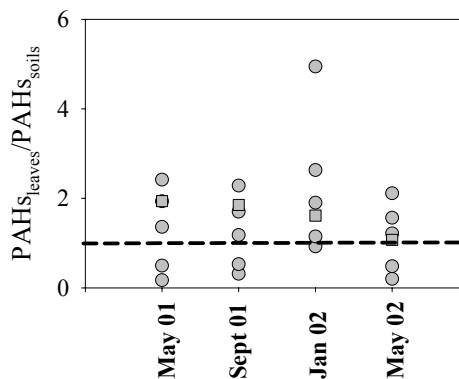

FIGURE 2
Coefficients of variation (numbers) of ΣPAH concentrations (circles) in the leaves and soils between the urban sites in May 2001, September 2001, January 2002, and May 2002.

FIGURE 3
Leaf/soil ratios of ΣPAH concentrations at the control (squares) and urban sites (circles) in May 2001, September 2001, January 2002, and May 2002.

The median concentrations of 2-, 3-, 4-, 5-, 6+7-ring PAHs showed that the main contribution to the total PAH value of the leaves was given by the 3-ring PAHs and for the soils by the 6+7-ring PAHs. Besides, at each site, the less abundant leaves` PAHs were those with 5-, 6+7-rings, whilst for the soils those with 2-rings (Fig. 4). Figure 4 reports the data concerning the sampling of May 2001, which is representative for all the other samplings.
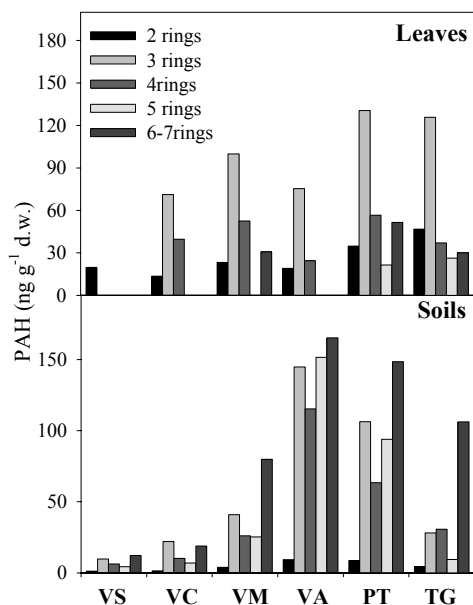

FIGURE 4
Median values of 2-, 3-, 4-, 5-, 6+7-ring PAHs at the control and urban sites in May 2001 (2-ring PAHs: naphthalene, acenaphtylene, acenaphthene, fluorene; 3-ring PAHs: phenanthrene, anthracene, fluoranthene, 2,3-benzofluorene; 4-ring PAHs: pyrene, benzo(c)phenanthrene, benzo(g,h,i) fluoranthene, benzo(b)-naphtha(1,2-d)thiophene, cyclopenta(c,d) pyrene, benzo(a) anthracene*, chrysene + triphenylene, benzo(b*+k*+j) fluoranthene, 1,3,5-triphenyilbenzene; 5-ring PAHs: benzo(e) pyrene, benzo(a) pyrene*, perylene, indeno(1,2,3-c,d) pyrene*, benzo(b)chrysene + picene, dibenzo(a,c) anthracene, dibenzo(a,h)anthracene*; 6+7-ring PAHs: benzo(g,h,i)perylene, anthanthrene, coronene).
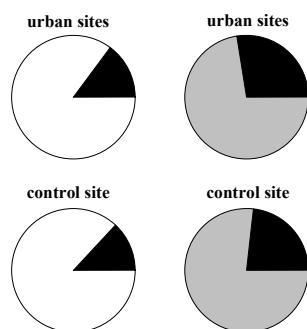*Below the instrumental detection limit

FIGURE 5
Proportions of the carcinogenic (benzo(a)anthracene, benzo(b+k+j)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene) PAHs (darker slices) to the ΣPAH value both in the leaves (white) and soils (grey) at the control and urban sites.

The carcinogenic PAH concentrations at each urban site, both for the leaves and soils, were on average higher than at the control site (Fig. 5). At the urban sites, the carcinogenic PAHs represented about 15% of the ΣPAH values in leaves and about 28% in the soils (Fig. 5), and at the control site the values were slightly lower. The carcinogenic PAH and ΣPAH concentrations were correlated (P<0.001) for the soils and leaves.

DISCUSSION AND CONCLUSION

The higher ΣPAH concentrations in *Q. ilex* leaves and surface soils at the urban sites compared to that at the control site indicate PAH accumulation both in the leaves and soils at all the sites of the urban area of Naples, as a consequence of the considerable air contamination [18]. Although soil is exposed to air PAH deposition for a longer time than *Q. ilex* leaves, the latter often show higher PAH accumulation. It is worth-noting that also at the control site PAH leaf concentrations were higher than PAH soil concentrations. *Q. ilex* leaves retained PAHs because of their thick layer of lipids and waxes [13], absorbing lipophilic compounds such as PAHs. The hairs of the leaves also play a role in capturing air particulates rich in PAHs [13, 15]. PAH accumulation in the soils, however, depends on the soil`s chemical-physical characteristics that may influence PAH retention and on microbial activities that control PAH decomposition [16].

The spatial trends both of leaf and soil ΣPAH concentrations observed among the urban sites would suggest a gradient of PAH air contamination. Various authors [8, 15, 18-20] observed a relationship between different degrees of air PAH contamination and soil and/or leaf PAHs. The non-correspondence at the urban sites between the spatial trends of leaf and soil ΣPAHs could be due to the different exposure time of both to air PAH contamination. Leaf time exposure is about 1 year and that of soil is longer. Besides, leaves intercept PAH air depositions, directly reflecting PAH air contamination. Soil PAH concentrations depend not only on deposition of air PAHs, but also on the presence of organic matter and microbial activities as well as site characteristics. Moreover, the surface soils collected at the base of the trees should be enriched in PAHs with all the particles deposited on the whole tree being washed off by rainfall [14]. The presence of organic matter in the surface soils could also affect the PAH concentrations because the organic soil layers adsorb the PAHs migrating downward and seem to capture PAHs migrating upward [8, 20]. Soil microbial activity would also seem to play a role in the persistence of PAHs in the soils as microorganisms transform some PAHs into other organic compounds [21, 22]. Indeed, it would seem that bacterial biodegradation starts from low-number ring PAHs. Hence, those PAHs with 5- and more rings are the more abundant [16]. Our findings fit with these considerations, showing an accumulation at all the sites of the 6/7- ring PAHs. The higher PAH soil

concentrations at the urban sites VA and PT, where there was no grass cover or herbaceous species, are probably due to direct deposition. At these sites, the scarce content of organic matter limits microbial activity, probably resulting in a conspicuous accumulation of the deposited PAHs, as demonstrated by the negative correlation found between ΣPAH concentrations and organic matter contents in the soils.

The wider temporal variation in ΣPAH concentrations of the leaves could be due to the briefer exposure time with respect to the soils. Soil ΣPAHs are values coming from the integration of PAH air contamination over a long-time exposure. Therefore, variations in concentrations over the investigated period are not really appreciable. The highest values of ΣPAH leaf concentrations in January may be attributable to the highest PAH air concentrations in winter because of the larger number of emission sources, the stability of atmospheric conditions and the presence of fewer degradation phenomena [4, 23]. In Naples, higher PAH air concentrations were measured in winter by Caricchia and co-workers [18].

The correspondence between the total and carcinogenic PAH concentrations both for the leaves and the soils at the urban and control sites should be taken into account because emissions of PAHs in the air may be hazardous for human health and for animals and plants [17].

In conclusion, although leaves appear to reflect seasonal deposition of PAHs and thus provide a more immediate indicator of PAH contamination, both *Q. ilex* leaves and surrounding soils allow us to evaluate PAH air spatial gradients. As biomonitors, leaves and soils might also add useful information about the effect of PAH air contamination on organisms.

ACKNOWLEDGMENTS

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