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Original article

PAHs accumulation on leaves of six evergreen urban shrubs: A field experiment

Guido Fellet ^{a,*}, Filip Pošćić ^a, Sabina Licen ^b, Luca Marchiol ^a, Rita Musetti ^a, Arianna Tolloi ^b, Pierluigi Barbieri ^b, Giuseppe Zerbi ^a

^a Department of Agriculture, Food, Animals and Environmental Sciences, University of Udine, via delle Scienze 208, I-33100 Udine, Italy

^b Department of Chemical and Pharmaceutical Sciences, University of Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy

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ABSTRACT

Air pollution refers to the occurrence of toxic substances in the atmosphere which results in detrimental effects to human beings and living environments. Among the most common atmospheric pollutants, Polycyclic Aromatic Compounds (PAHs) are the most common substances originated by vehicles. The aim of the study was to investigate the accumulation of 16 PAHs in leaves of six evergreen shrubs frequently used in Italy for urban landscaping (*Elaeagnus x ebbingei*, *Ilex aquifolium*, *Laurus nobilis*, *Ligustrum japonicum*, *Photinia x fraserii* and *Viburnum lucidum*). The study was conducted in two sites: a public park and a high traffic square. Six samplings were performed in a period of 26 months during Winter. The plants tolerance was investigated considering leaf-extract pH, total chlorophyll, leaf ascorbic acid content and relative leaf water content. A morphological leaf characterization was also carried out considering stomatal density and cuticle width. Phenanthrene, fluoranthene, fluorene and pyrene were the major compounds yielded in the plant leaves accounting for about 83% of ΣPAHs, the contributions being 53%, 11.3%, 10.5% and 8.5%, respectively. Such compounds are related to vehicular emissions. The analysis of the PHAs accumulation and the evaluation of the plants tolerance also revealed that the efficiency in trapping 5- and 6-rings potentially carcinogenic PAHs such as benzo(b)fluoranthene, benzo(k)fluoranthene in plant leaves is highest for *Elaeagnus x ebbingei*, *L. japonicum* and *L. nobilis* while *Elaeagnus x ebbingei* and *Photinia x fraserii* resulted to be those most tolerant to air pollution. The role of plants in mitigating traffic pollution is confirmed.

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1. Introduction

Air pollution refers to the condition in which the existence of toxic substances in the atmosphere, generated by various human activities and natural phenomena, results in damaging effects on the welfare of human beings and the living environment (Omasa et al., 2002). In 2013 the International Agency for Research on Cancer classified outdoor air pollution as carcinogenic to humans (Straif et al., 2013).

Among the most common atmospheric semi-volatile compounds, PAHs are ubiquitous pollutants from both natural and

anthropogenic sources (Cheruiyot et al., 2015). Natural sources include forest fires, volcanic eruptions and degradation of biological materials. Anthropogenic sources are the most widespread contributors (Lesage and Jackson, 1992), including vehicle and furnace exhausts, coal and oil-fired power plants, gasification/liquefaction of fossil fuels, coke and asphalt production, waste incinerators, aluminum smelting and gas and oil flare operations. PAHs can partition among air and solid phases, with lighter congeners being more volatile and heavier terms essentially adsorbed on condensed phases; most toxic terms are usually adsorbed on particulate matter (Zhang et al., 2007; Liu et al., 2009) and in urban environments they can easily be inhaled into residents' lung. Some PAHs are well-known for their mutagenic, carcinogenic and teratogenic activities (Boström et al., 2002; Shi et al., 2010). The US EPA (1989) identified 16 priority PAHs that have become targets of environmental investigations worldwide (Peng et al., 2012). The

* Corresponding author. Tel.: +39 432 558666; fax: +39 432 558603.

E-mail address: guido.fellet@uniud.it (G. Fellet).

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EU Directive 2004/107/EC identified benzo(a)pyrene as a marker of the carcinogenic risk of PAHs.

In order to mitigate the health impacts from this type of pollution, urban forests and greenbelts have received increasing attention for the development of efficient strategies with the objective of improving air quality. Several studies have demonstrated the positive influence of trees, shrubs and infrastructures such as green walls and green roofs, on the interception of particulate matter and abatements of contaminant levels in urban environments (Currie and Bass, 2008). The study of how to exploit this potential is the challenge for urban planners and policy-makers, while researchers must provide more precise data on the potential of many plant species under the specific conditions of use. Models such as UFORE and others (Tallis et al., 2011; Nowak et al., 2013) are available to accurately predict the concentrations of some pollutants both before and after green space establishment. It has been demonstrated that leaves of trees and shrubs can efficiently trap air-borne pollutants, removing them from the urban atmosphere (Nowak et al., 2013). A number of plant species have already been studied as PAHs scavengers (Simonich and Hites, 1995; Slaski et al., 2000; Ahammed et al., 2012). As these compounds are lipophilic the most important pathways through which airborne PAHs can enter plants are (i) gas-phase and particle-phase deposition onto the waxy cuticle of the leaves or (ii) by uptake through the stomata. The interception process depends on several biotic and abiotic factors, including the phase state of PAH, leaf surface area, lipid concentration in plant tissue, octanol–air partitioning coefficient and ambient temperature (Slaski et al., 2000). Less important is root uptake for the fraction of pollutants deposited in the soil (Simonich and Hites, 1994).

When exposed to air pollutants, most plants experience physiological changes before exhibiting visible damage to leaves (Govindaraju et al., 2012). Some basic biochemical parameters such as membrane permeability (Farooq and Beg, 1980), glutathione concentration (Hoque et al., 2007) and peroxidase activity (Lee et al., 2007) were used to estimate plant sensitivity and tolerance to air pollutants. De Nicola et al. (2011a) demonstrated that in urban environments, the photosynthetic performance with aging of leaves of *Quercus ilex* strongly decreased and that this was mainly due to impaired CO₂ assimilation caused by stomata occlusion. Other studies showed that air pollutants could also affect ascorbic acid, chlorophyll content, leaf-extract pH and relative water content (RWC), as well as morphological alterations and occurrence of DNA damages as reported by Arena et al. (2014) in *Q. ilex*. More specifically, phytotoxicity of PAHs to for several species is well documented as inhibition of germination, growth and photosynthesis as well as the effect of the N-heterocyclic derivatives (Pašková et al., 2006). Studies on the effects of PAHs on plants demonstrated morphological damage and metabolic alterations such as oxidative stress, cell death, upregulation of antioxidant systems (Liu et al., 2009). It is likely that species-specific defensive adaptations exist but they are not yet known (Weisman et al., 2010).

The Air Pollution Tolerance Index (APTI) is a parameter developed by Singh and Rao (1983), calculated based on four biochemical parameters (ascorbic acid, chlorophyll content, leaf-extract pH and RWC) and used by landscapers to select plant species tolerant to air pollution (Seyyednjad et al., 2011; Radhapriya et al., 2012).

So far, field studies investigating the ability of plants to absorb airborne PAHs have been conducted by sampling and analyzing plant fractions (mainly tree leaves) collected in two scenarios. In the first, pre-existing plants growing in urban and periurban areas, along roadsides, public parks or private gardens were sampled (Lodovici et al., 1998, 2003; Howsam et al., 2000; Lehndorff and Schwark, 2004; Singh and Verma, 2007; Wang et al., 2008; Lehndorff and Schwark, 2009; De Nicola et al., 2011b; Papa et al.,

2012; Noth et al., 2013; Terzaghi et al., 2013). In the second scenario, the plants were sampled in the surroundings of disused or still working industrial sites (Sharma and Tripathi, 2009; Rodriguez et al., 2010, 2012; Wang et al., 2012). So, in both cases plants were exposed to air pollution for a long time, whereas we studied PAHs accumulation in evergreen shrubs transplanted to the experimental sites a couple of months before starting the sampling. The choice to investigate shrub species rather than trees was due to their faster growth rate and the fact that such plants usually grow within the height range of 1–3 m from the ground, which is very close to vehicle emissions.

The aims of this study were to: (i) investigate the concentration of PAHs in the leaves of evergreen shrubs grown in Italy in the public and private gardens exposed to outdoor air pollution in urban environments, (ii) verify the relationship between the increase of concentration of PAHs in the leaves and exposure time so to select the more suitable one for environmental biomonitoring and (iii) evaluate the APTI. In studying goals, the most suitable shrub for green urban spaces to efficiently moderate atmospheric pollution by PAHs can be selected. Given that the molecular weight of PAHs is related to their distribution among the gaseous phase and the particle-bound fraction of the atmosphere (low molecular weight PAHs are predominantly present in the gaseous phase while higher molecular weight PAHs are linked to the large particle fractions) (Desalme et al., 2013), the present study discriminates the pollutants according to the number of rings of which they are formed.

2. Materials and methods

2.1. Site description and sample collection

The study, that had a duration of 26 months (November 2009–January 2012), was conducted within the urban area of Udine, N Italy (46°04'42"N, 13°14'16" E and 113 m above sea level; mean annual rainfall = ca.1210 mm; mean temperature = 13 °C) and was part of a project funded by the Italian Ministry of Agriculture, Food and Forestry (MIPAAF, Decree 11056/7643/09). This work focused on the analysis of 16 priority PAHs based on the US EPA list. The minimum, maximum and mean concentrations for each PAH in each site for each species over the studied period are reported in Table 1 (A and B).

Three-year-old potted specimens (4 plants for each species, at each site; n = 4) of 1.5 m tall *Elaeagnus x ebbingei*, *Ilex aquifolium*, *Laurus nobilis*, *Ligustrum japonicum*, *Photinia x fraserii* and *Viburnum lucidum* were transplanted in November 2009 in a public park (CAIR) and a high traffic square (POSO). The experimental sites were chosen for their proximity to Regional Environmental Protection Agency (ARPA-FVG) air quality monitoring stations equipped for daily detection of the concentration of PM₁₀ (Fig. 1). Replicates were assigned a random position within the experimental area of the two sites.

Focusing on colder periods of the year when atmospheric dispersion is less efficient, air pollution provokes alerts and both the photo- and ozone-degradation of organic contaminants is relatively unimportant (Masiol et al., 2013), six samplings were taken on the following dates: 10 February 2010 (F10), 14 October (O10), 16 December 2010 (D10), 10 February 2011 (F11), 10 November (N11) and 12 January 2012 (J12).

2.2. Morphological characterization of plant leaves

One-year-old leaves of each species (n = 8) were collected for morphological characterization. Epidermal impressions were obtained applying nail polish to the abaxial surface of leaves. Stomatal

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