Bull. Environ. Contam. Toxicol. (1992) 48:809–814

Environmental Contamination and Toxicology

Polycyclic Aromatic Hydrocarbons in Olive Fruits as a Measure of Air Pollution in the Valley of Florence (Italy)

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Plants have often been used for monitoring air pollution, such as Tradescantia for detecting mutagenic chemicals (Ma et al.,1984), or mosses which are bio-accumulators of heavy metals (Strachan and Glooschenka, 1988; Harrison and Chirgawi, 1989). Mosses have also been used as indicators of pollution from hexachlorobenzene and polycyclic aromatic hydrocarbons (PAH) (Thomas, 1986). PAH are present in most crops (Dennis et al. 1983), and are deposited on the foliar surface of plants exposed to polluted air. Plants grown in heavily polluted environments have a higher concentration of PAH than those growing in clean environments (Grimmer and Hildebrandt, 1965), and plants grown in cabinets with filtered air have a very low concentration of PAH (Grimmer and Duevel, 1970). Alimentary oils have high concentrations of PAH (Howard et al 1966) due to crop exposure to air pollutants and a high solubility of PAH in oils.

PAH are important initiators of some human cancers and their monitoring is believed to be important for public health. Most Italian towns are heavily polluted by car exhaust and industrial sources, and a high concentration of PAH has been reported in the air particulate of urban areas (WHO, 1988).

On the basis of these premises we though it of interest to determine the concentration of some PAH in the olive fruits of trees growing in the valley of Florence (Italy), to establish if this approach could be useful for monitoring air pollution by PAH.

MATERIALS AND METHODS

Olives were picked before harvest time (late November) from 34 locations distributed in a radius of about 15 Km from Florence. Some olive trees were located within the city limits (Fig.1). The locations varied in terms

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of proximity to heavy traffic zones and in altitude. Some were located in hill areas close to the city but far from major roads (site 1,2 and 3). Florence is located in the narrow valley of the Arno river flowing westward, the hills running parallel to the river.

Samples of 100 g of olives were hand picked, rinsed with 100 ml of distilled water, dried on filter paper and kept at -20° C until analysis, which was done within 60 days. Olives were extracted in a reflux funnel for 3.5 h in 140 ml of methanol:water (9:1 v:v) made basic with 8 g of NaOH. As an internal standard we added 40 ng of 3-methylcholanthrene at the beginning of the procedure. The extraction medium was filtered through glass wool, washed with 40 ml of the same methanol:water solution and extracted twice with 70 ml of iso-octane discarding the methanol:water phase. The iso-octane phases were pooled, washed with 140 ml of methanol:water (1:1 v:v). We then extracted the isooctane phase with N,N-dimethylformamide:water (9:1 v:v) discarding the iso-octane phase. The N,N-dimethylformamide:water phase was diluted with an additional 140 ml of water, extracting it again with 140 ml of iso-octane. The final iso-octane solution was dehydrated with 5 g of anhydrous sodium sulfate on a plastic column and concentrated to 2 ml with a rotary evaporator at 45°C.

The concentrated extract was passed through a silica column (3 g) pre-washed with 20 ml of chloroform and iso-octane in sequence, eluted with 20 ml of iso-octane and taken to dryness in a nitrogen stream. With this procedure we removed interfering components. The residue was finally dissolved in 200 µl of methanol: acetonitrile (9:1, v:v) and analyzed by HPLC. The percent recovery was 36.9+/-12 (SD). We used a C18 Supelco reverse phase column (i.d:0.46; length: 25 cm; particle size: 5 µm) and as eluting solution methanol: water (60:40 v:v) with a flow rate of 1.2 ml. Separation was obtained with an isocratic of 40% acetonitrile in water for 5 min, followed by a linear gradient in which the acetonitrile concentration increased from 40 to 90 % in 20 min. We used a fluorometric detector (excitation wavelength: 290 nm; emission wavelength 430 nm) and a Hitachi integrator for the determination of peak areas. The values were expressed as µg of PAH/Kg olive wet weight. We determined benzo(a)pyrene, fluoranthrene benzo(ghi)perilene, benzo(a)anthracene and pyrene.

Repeated samples of 24 h air were collected with a high volume pump set at 1 m^3/min. About 1,600 m^3 of air

particulate were collected on glass wool filters (P/N-G810, 20.3x25.4 cm, General Metal Work Inc. Ohio, USA), that was later extracted with 250 ml cyclohexane for 12 h in a Soxlet apparatus. The extract was taken to dryness in a rotary evaporator, resuspended in a small volume of methylene chloride:methanol (1:1), and analyzed with the same column and detector described in the previous section using as carriers acetonitrile: water (40:60) and a linear gradient up to 100% of acetonitrile, with a flux of 1 ml/min.

RESULTS AND DISCUSSION

The levels of benzo(a)pyrene and of the sum of the five PAH in olives taken from different locations are shown in Fig.1.

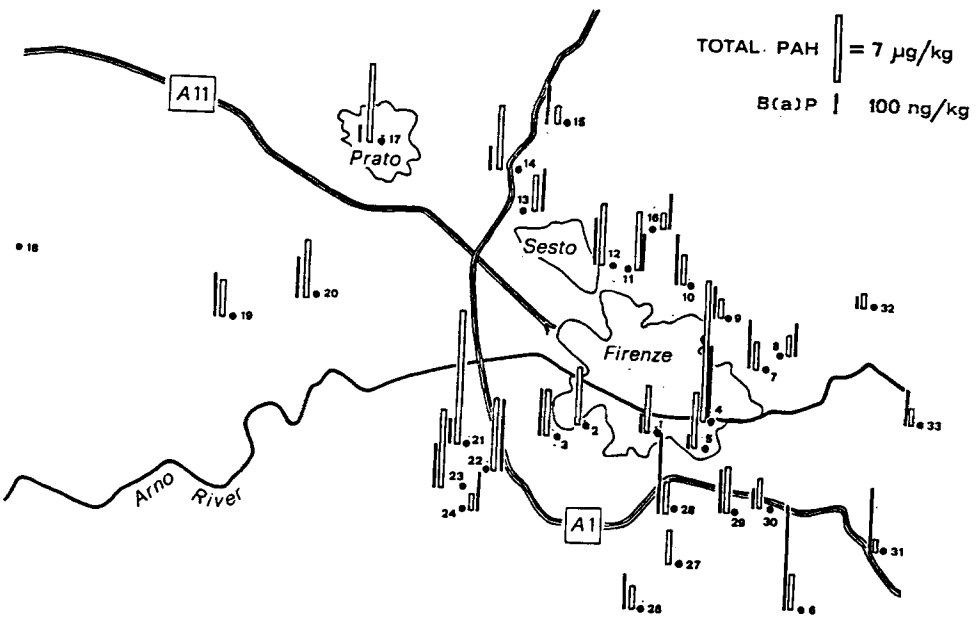

Figure 1. Sampling sites in the valley of Florence. Bar height in each site refers to the levels of benzo(a)pyrene (ng/Kg of olives) and to the sum of 5 PAH (benzo(a)pyrene, benzo(a)anthracene, pyrene, fluoranthrene and benzo(ghi)perilene)(µg/Kg).

We always found measurable amounts of PAH in the olives, some samples having high concentrations of PAH (up to 15 µg/Kg, as a sum of 5 PAH), and some others very low (between 1 and 3 µg/Kg).

The main source of PAHs in the valley is car exhaust, since most private and public heating systems utilize methane as fuel and big industries and power plants using fossil fuel as well as garbage incinerators are not present in the valley.

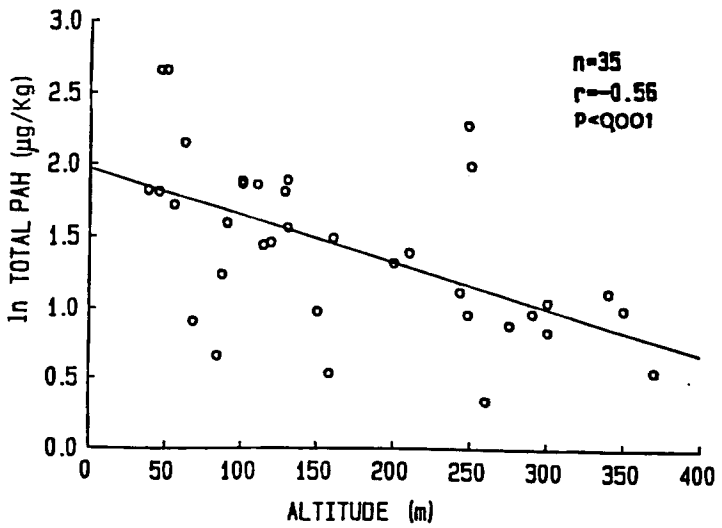

Figure 2. Correlation between altitude and the ln of the olive concentration (g/kg) of the 5 PAH of fig.1.

On these premises we expected to find higher values of PAH in most of the downtown locations (enclosed in the continuous line of the city borders of Firenze and Prato of Fig.1) and around the main north-south highway (A1 in Fig.1). On the contrary olives of some downtown locations (like site 1, Forte Belvedere, located on a hill) and some hillside locations around the highway (sites 24,27,29,30 and 31), had low concentrations of PAH. We, therefore, correlated the ln of the concentration of the five PAH (Fig.2) to the elevation of the different sampling sites.

It was apparent that PAH concentration showed an exponential decrease with altitude. We also obtained measurements of PAH in air particulate in three different sites and we compared the average values of PAH in the air during the period of olive fruit growth (April-November) to the concentration in olives near the air sampling stations (Fig.3).

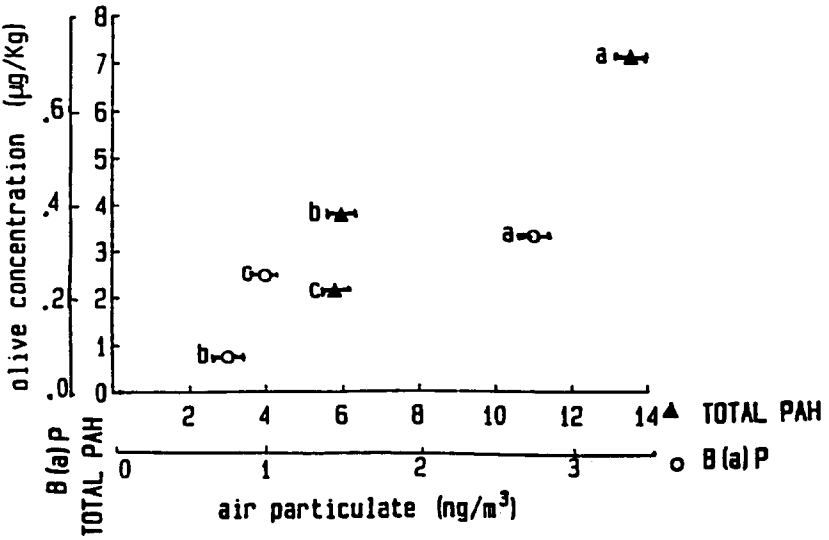

Figure 3. Correlation between total PAH and B(a)P levels in 24 h air particulate and values in nearby olives. Air sampling sites were (a) downtown Florence (compared to sites 4-5 of Fig.1), (b) downtown Prato (compared to site 17) and (c) a hill rest stop of highway A1 (com-pared to sites 28-29-30). Mean values +/-SE of (a) 28 air determinations (24 h), (b) 10 determinations and (c) 8 determinations in the period April-November.

Air particulate levels of total PAH and benzo(a)pyrene seemed to be correlated to values measured in the olives, confirming what Grimmer and Hildebrandt (1965) observed for other plants. Olives are matrices of water and oil that bear similarities to the elementary composition of animal tissues. In this respect the accumulation of PAH in olives in a certain area might give useful information on the deposition of PAH and might be a good indicator for correlating PAH levels with biological effects. In the valley of Florence the deposition of PAH seems to be more related to altitude than to proximity to heavy automotive traffic.

Acknowledgements. Supported by the Regional Government of Tuscany and the Ministry of Public Instruction of Italy.

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Received March 15, 1991; accepted December 30, 1991.