DOI:10.1021/jf9011054

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Monitoring of Polycyclic Aromatic Hydrocarbons in Bees (*Apis mellifera*) and Honey in Urban Areas and Wildlife Reserves

MONIA PERUGINI,*,[†] GABRIELLA DI SERAFINO,[†] ALESSANDRA GIACOMELLI,[†] PIOTR MEDRZYCKI,[‡] ANNA GLORIA SABATINI,[‡] LIVIA PERSANO ODDO,[‡] ENZO MARINELLI,[‡] AND MICHELE AMORENA[†]

[†]Department of Food Science, University of Teramo, Viale Crispi 212, 64100 Teramo, Italy, and [‡]Council for Research and Sperimentation of Agriculture (CRA), Via Nazionale 82, 00184 Roma, Italy

The honeybee is a good biological indicator that quickly reflects chemical impairment of the environment by its high mortality and the presence of pollutants in its body or in beehive products. In this work the honeybee (*Apis mellifera*) and honey were used to detect the presence of polycyclic aromatic hydrocarbons (PAHs) in several areas with different degrees of environmental pollution. All sampling sites showed the presence of PAHs. Benzo(*a*)pyrene was never detected. Fluorene, phenanthrene, anthracene, fluoranthene, benz(*a*)anthracene, benzo(*b*)fluoranthene, and benzo(*k*)fluoranthene were the PAHs detected in bees, whereas the honey contained only phenanthrene, anthracene, and chrysene. Phenanthrene showed the highest mean values in honeybees and honey. Independent from the season and location the pattern of PAHs in honeybees and honey was dominated by the presence of the lowest molecular weight PAHs. Furthermore, the mean PAH concentrations in honey samples were lower than those reported in honeybees, and no positive correlation was found between the compounds detected in bees and those in honey.

KEYWORDS: Polycyclic aromatic hydrocarbons; *Apis mellifera*; bioindicators; honey; monitoring

Downloaded by monia perugini on August 26, 2009 | http://pubs.acs.org
Publication Date (Web): July 23, 2009 | doi: 10.1021/jf9011054

INTRODUCTION

Several ecological, ethological, and morphological characteristics have made the honeybee (*Apis mellifera*) a reliable ecological detector that can be a useful method for the determination of levels of anthropogenic contamination in large areas (*1*−*3*). Honeybees are known to collect bioavailable contaminants, sampling most of the environmental sectors such as vegetation, water, soil, and air. Whereas mechanical fixed position instruments give punctiform values, honeybees provide data over the area covered during foraging. They have great mobility and are continuously exposed to pollutants from atmospheric pollution and present in the area surrounding the hives (*4*, *5*). They are social insects nesting in colonies typically comprising a single queen, drones, and numerous workers. Forager bees commonly fly within 1.5 km of their hive, but they can range over long distances, even up to 10 km depending on their need for food and its availability (*6*). During the foraging flights they pick up airborne particles with their body hair while collecting pollen and nectar from flowers. The forager brings samples into the hive by gathering nectar and pollen from flowers, honeydew from the aphids of infested plants, and water from wells and pools. They also collect dust of various origins on their body hair. Each forager completes 12−15 foraging trips a day. On return to the hive, the nest-mates in the hive fan the air furiously with their wings, releasing the pollutants into the hive. Occasionally, bees also accumulate residues in their bodies, by stockpiling contaminants on direct exposure to residual pesticides. Thus, these insects are unbiased samplers that can be used to detect organic and inorganic pollutants in the environment; more recently, the services of bees have been extended in the routine monitoring of volatile and semivolatile organic contaminants. The bee family can be easily breed and, because they show a high reproduction rate, are also numerous, so it is generally possible to collect a sufficient number of samples. As such, honeybees and honey can supply a suitable amount of biological material to be sampled and analyzed throughout the year (*7*, *8*).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous chemical compounds consisting of at least two or more fused aromatic rings of carbon and hydrogen atoms. They are generally formed as the result of incomplete combustion of organic material (*9*), and the majority of PAH deposition is anthropogenic (*10*). Forest fires, domestic heating, combustion of fossil fuels such as gasoline, coal, and diesel fuel, industrial activities such as petroleum refining processes and catalytic cracking, rural and urban sewage sludge, smoking food processes, and tobacco and cigarette smoke represent only a few PAH sources. The chemical properties of PAHs depend on their number of rings and molecular mass. Because of these properties, PAHs in the environment are found primarily in soil, sediment, and oily substances, as opposed to in the water or air. However, they are also a component of concern in particulate matter suspended

*Author to whom correspondence should be addressed [telephone +39(0)861266988; fax +39(0)861266987; e-mail mperugini@unite.it].

Published on Web 07/23/2009
© 2009 American Chemical Society

in air. They are known for their carcinogenic and mutagenic properties and for being responsible of background level contamination in environmental matrices (*11*).

PAH toxicity is very structurally dependent, with compounds varying from being nontoxic to being extremely toxic. Dihydrodiols and epoxide derivatives, products of the liver by PAH metabolism, form covalent adducts with DNA and proteins that begin a mutagenic process in the cells. The most studied individual PAH compound is benzo(*a*)pyrene (BaP), but it is only 1 of at least 100 PAHs that have been identified in the air and that in the environment occur as complex mixtures. The quantification of PAHs is particularly advantageous when their profiles can be correlated with sources and effects. The evaluation of PAHs across a large spatial area, by traditional methods, would require expensive sample effort, but the potential to use bees and honey as indicators of possible environmental pollution takes advantage of the possibility to monitor large areas with extremely low costs.

The purpose of this study was the application of honeybees and honey as biological indicators to assess the levels of PAH atmospheric pollution. This research involves direct measurements of PAH concentrations in the tissues of bees and in honey to detect variations in PAH concentrations among samples collected from sampling sites characterized by different environmental impact and to evaluate the long-term variations of the level of anthropogenic contamination in large areas. The site surveys were performed in two regions having different degrees of environmental pollution: Abruzzi, known for unpolluted areas such as wildlife reserves and agricultural-forest sites, and Latium, characterized by more industrialized and anthropogenic areas, all of which are near Rome. Furthermore, we investigated the relationship between the PAH levels reported in the bees in comparison to honey contamination, verifying the possibility to consider bees as a vector of contamination.

Downloaded by monia perugini on August 26, 2009 | http://pubs.acs.org
Publication Date (Web): July 23, 2009 | doi: 10.1021/jf9011054

MATERIALS AND METHODS

Reagents and Materials. PAH-mix9 (100 ng μL^{-1} in acetonitrile) was supplied by Dr. Ehrenstorfer, Reference Materials (Augsburg, Germany), stored at 4 °C, and used for the preparation of working standard solutions. Acetone and hexane (for the analysis of pesticide residues), acetonitrile for HPLC, water plus for HPLC, and anhydrous sodium sulfate crystals were provided by Carlo Erba (Milan, Italy).

Sample Collection. The research was run from May to October 2007. The apiaries were located in two different Italian regions, Abruzzi and Latium, within urban areas or in wide countryside areas. Six sampling sites (2−7) were selected as unpolluted areas because they were within wildlife reserves and a considerable distance from polluted areas; one site (8) was located next to Ciampino airport, characterized by intense air traffic and motor vehicle circulation; and the last site (1) was located in a moderately polluted area, near a small road and an incinerator. Each sampling station consisted of three healthy beehives, Dadant-Blatt type, at 10 combs.

Sample collection was carried out each month in the late morning, without the use of a smoker to avoid the risk of external PAH contamination. Forager bees were caught at the entrance of the hive and immediately stored in dry ice. The fresh honey (with a humidity of > 18%) was collected directly from the uncapped honeycomb, to ensure that the honey was a product in the same month of the sampling, and stored at 4 °C until the beginning of the analytical procedure.

Sample Honeybee Analysis. The bees were cleaned of pollen and drones before the analysis. Each sample was divided in two aliquots; the first of about 2 g was used to determine the moisture, and the other was lyophilized. About 1 g of lyophilized sample was homogenized in a glass mortar with 5 g of Extrelut (Extrelut NT, Merck, Darmstadt, Germany) and then extracted with a mixture 1:1 of *n*-hexane/acetone by means of an accelerated solvent extractor (ASE 100, Dionex Corp., Sunnyvale, CA) at the following conditions: oven temperature, 100 °C; static time, 5 min; static cycles, 2; flush volume, 60% of extraction cell volume (34 mL); nitrogen purge, 1 MPa for 60 s. The solvent was filtered and evaporated to dryness in a rotary evaporator at 35 °C. The extracts obtained from bee samples were dissolved with 1 mL of acetonitrile before analysis.

Table 1. Limit of Detection (LOD), Limit of Quantification (LOQ), Wavelength, Percentage of Recovery, and Linearity of PAHs in Honeybees

compd	wavelength ($\lambda_{ex/em}$, nm)	recovery (%)	linearity (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
FL	280−330	73 ± 11	0.9993	0.05	0.08
Phe	246−370	82 ± 13	0.9981	0.10	0.12
A	250−406	85 ± 6	0.9982	0.02	0.05
F	280−450	87 ± 7	0.9960	0.07	0.11
PY	270−390	93 ± 6	0.9996	0.49	0.62
BaA	265−380	93 ± 8	0.9998	0.12	0.15
Ch	265−380	92 ± 9	0.9995	0.16	0.18
BbF	290−430	96 ± 12	0.9990	0.05	0.06
BkF	290−430	98 ± 5	0.9965	0.01	0.02
BaP	290−430	95 ± 6	0.9992	0.05	0.08
DBahA	290−410	93 ± 9	0.9992	0.05	0.07
BghiP	290−410	94 ± 7	0.9963	0.20	0.28
IP	300−500	87 ± 11	0.9852	0.21	0.26

Sample Honey Analysis. In the laboratory wax particles were removed and about 5 g of honey, mixed with 8 g of Extrelut, was extracted using a mixture 1:1 of *n*-hexane/acetone by means of an ultrasonic bath for 20 min. The ultrasonic cycle was repeated twice. The extracts were passed through a filter containing anhydrous sodium sulfate and evaporated to dryness at 35 °C under a flow of nitrogen. The samples were dissolved with 1 mL of acetonitrile before analysis.

Chromatography Conditions. Quantitative analysis of PAHs was carried out with a high-performance liquid chromatography (HPLC) apparatus equipped with a 20 μL loop and a fluorescence detector (ProStar 363, Varian, Palo Alto, CA) with variable excitation and emission wavelengths. The software used was Star Chromatography Workstation version 5.2 (Varian).

PAHs were separated at ambient temperature using a C18 Envirosep-pp column (Phenomenex, Torrance, CA; 12.5 cm × 4.60 mm, particle size = 3 μm) and a gradient elution program with a flow rate of 1.4 mL/min. The initial mobile phase was 65% acetonitrile and 35% HPLC water for 8 min, which was then gradually changed to 100% acetonitrile over 1 min, held at 100% for 11 min, and then decreased to initial phase (65:35%).

The investigated PAHs were acenaphthene (AP), fluorene (FL), phenanthrene (Phe), anthracene (A), fluoranthene (F), pyrene (PY), benz(*a*)-anthracene (BaA), chrysene (Ch), benzo(*b*)fluoranthene (BbF), benzo-(*k*)fluoranthene (BkF), benzo(*a*)pyrene (BaP), dibenz(*a*,*h*)anthracene (DBahA), benzo(*ghi*)perylene (BghiP), and indeno(1,2,3-*cd*)pyrene (IP). PAHs were identified on the basis of retention time, and quantification was performed by an external standard method.

The detection limit (LOD) and the quantification limit (LOQ) were determined according to the standard deviation method. A series (n = 10) of blank samples, containing no analyte but with a matrix identical to that of the samples analyzed, were injected in triplicate, and the mean blank value and the standard deviation (SD) were calculated. The LOD was the mean blank value plus 3 SD, whereas the LOQ was the mean blank value plus 6 SD. The precision and accuracy of the method were assessed using nine determinations over a minimum of three concentration levels. Analyte recoveries were determined by using honeybees and honey samples spiked with solutions of the PAH standard (PAH-mix9 in acetonitrile) to reach a final concentration of 10, 25, or 50 ng mL^{-1} in each sample. The external standard multipoint calibration technique was used to determine the linear response interval of the detector, and the working standard solutions were of 1, 5, 10, 25, and 50 ng mL^{-1} in acetonitrile. The response obtained was linear across the assayed concentration range. **Tables 1** and **2** summarize the analytical results for honeybees and honey, respectively.

Statistical Analysis. For all samples with concentrations below the limit of quantification, zero was used in the calculation. Normality of data of PAHs, calculated on a fresh basis, was assessed by means of the Kolmogorov−Smirnov test. In some compounds data were not normally

Downloaded by monia perugini on August 26, 2009 | http://pubs.acs.org
Publication Date (Web): July 23, 2009 | doi: 10.1021/jf9011054

distributed; therefore, they were log transformed and normality was assessed again. Then, analysis of variance (ANOVA) was performed to detect significant differences among groups (according to sampling site and season) with the statistical package SPSS 14.0.2 (SPSS Inc., Chicago, IL). Moreover, possible correlation between PAHs in bees and in honey was assessed by means of Pearson and Spearman correlations with the same statistical package.

Table 2. Limit of Detection (LOD), Limit of Quantification (LOQ), Wavelength, Percentage of Recovery, and Linearity of PAHs in Honey

compd	wavelength ($\lambda_{ex/em}$, nm)	recovery (%)	linearity (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
FL	280−330	80 ± 13	0.9993	0.05	0.08
Phe	246−370	85 ± 13	0.9981	0.05	0.08
A	250−406	86 ± 4	0.9982	0.02	0.05
F	280−450	85 ± 7	0.9960	0.10	0.13
PY	270−390	88 ± 6	0.9996	0.49	0.53
BaA	265−380	89 ± 5	0.9998	0.10	0.13
Ch	265−380	90 ± 10	0.9995	0.05	0.06
BbF	290−430	92 ± 8	0.9990	0.04	0.06
BkF	290−430	94 ± 3	0.9965	0.01	0.02
BaP	290−430	94 ± 6	0.9992	0.04	0.06
DBahA	290−410	93 ± 4	0.9992	0.06	0.09
BghiP	290−410	96 ± 6	0.9963	0.17	0.21
IP	300−500	90 ± 11	0.9852	0.19	0.21

RESULTS AND DISCUSSION

The PAH concentrations in bees are reported in **Table 3**, whereas **Table 4** shows the results for honey samples. **Figure 1** shows a typical chromatogram of a honey sample naturally contaminated and a chromatogram of the same honey sample fortified with the mixture of PAHs, whereas **Figure 2** shows the standard of PAHs at 10 ng mL^{-1}.

Few investigated samples were free of PAHs, but most of the data on honeybees and honey showed low PAH levels with maximum values never higher than 10 ng g^{-1} of fresh weight for the bees and 3 ng g^{-1} of fresh weight for the honey. BaP, which represents the most potentially carcinogenic PAH (*12*, *13*), was never detected. FL, Phe, A, F, BaA, BbF, and BkF were the PAHs detected in bees, whereas the honey showed only Phe, A, and Ch. Phe was found in 87% of the honeybee samples and showed the highest mean values at each sampling station. It was also the most representative PAH in the honey (49%).

Independent from the season and location, PAH composition pattern in honeybees was dominated by the presence of the lowest molecular weight PAHs and, in particular, the most representative PAHs were those with three rings (93%) followed by those with four rings (6%) and five rings (1%). In honey, PAHs with five rings were never detected and 57% of reported compounds had three rings.

Table 3. Range, Mean, and Standard Error Values (SEM) of PAHs and Σ PAHs (Nanograms per Gram of Wet Weight) in Honeybees

sampling site		FL	Phe	A	F	BaA	BbF	BkF	Σ PAHs
1	mean	0.22	1.52	0.03	0.32	0.07	0.01	0.02	2.18
	SEM	0.146	0.41	0.02	0.12	0.05	0.01	0.01	0.71
	min	<LOD	0.18	<LOD	<LOD	<LOD	<LOD	<LOD	0.18
	max	1.56	4.72	0.22	1.09	0.45	0.07	0.18	8.22
2	mean	0.29	1.34	0.01	0.15	<LOD	<LOD	<LOD	1.80
	SEM	0.21	0.38	0.00	0.07			0.00	0.59
	min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.05
	max	3.17	5.96	0.05	0.84	<LOD	<LOD	0.02	9.12
3	mean	0.10	1.28	0.06	0.19	<LOD	<LOD	0.01	1.59
	SEM	0.05	0.20	0.00	0.10	0.00		0.01	0.31
	min	<LOD	0.28	<LOD	<LOD	<LOD	<LOD	<LOD	0.28
	max	0.50	2.27	0.05	0.95	0.05	<LOD	0.09	3.52
4	mean	0.45	2.13	0.01	0.38	<LOD	0.05	0.03	3.04
	SEM	0.23	0.40	0.00	0.15		0.03	0.02	0.70
	min	<LOD	0.44	<LOD	<LOD	<LOD	<LOD	<LOD	0.44
	max	3.04	6.02	0.05	1.65	<LOD	0.32	0.27	9.28
5	mean	0.08	1.30	0.01	0.23	<LOD	0.01	<LOD	1.62
	SEM	0.04	0.18	0.00	0.11		0.01	0.00	0.29
	min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	max	0.50	2.57	0.04	1.12	<LOD	0.10	0.02	3.73
6	mean	0.07	1.75	<LOD	0.05	<LOD	<LOD	<LOD	1.87
	SEM	0.02	0.49	0.00	0.03	0.00		0.00	0.50
	min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	max	0.30	9.24	0.04	0.42	0.05	<LOD	0.04	9.24
7	mean	0.06	0.10	<LOD	<LOD	<LOD	<LOD	<LOD	1.05
	SEM	0.02	0.13	0.00					0.15
	min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	max	0.29	1.84	0.04	<LOD	<LOD	<LOD	<LOD	2.04
8	mean	0.09	1.38	0.01	0.06	<LOD	0.12	0.01	1.67
	SEM	0.04	0.26	0.01	0.03		0.11	0.01	0.34
	min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	max	0.52	3.63	0.09	0.47	<LOD	1.82	0.10	4.68

Downloaded by monia perugini on August 26, 2009 | http://pubs.acs.org
Publication Date (Web): July 23, 2009 | doi: 10.1021/jf9011054

Table 4. Range, Mean, and Standard Error (SEM) Values of PAHs and Σ PAHs (Nanograms per Gram of Wet Weight) in Honey

sampling site		Phe	A	Ch	Σ PAHs
1	mean	0.30	0.06	0.38	0.74
	SEM	0.15	0.03	0.17	0.24
	min	<LOD	<LOD	<LOD	<LOD
	max	1.45	0.31	1.49	2.42
2	mean	0.48	0.09	<LOD	0.58
	SEM	0.20	0.04		0.24
	min	<LOD	<LOD	<LOD	<LOD
	max	2.05	0.41	<LOD	2.46
3	mean	0.13	0.02	0.01	0.15
	SEM	0.10	0.02	0.01	0.12
	min	<LOD	<LOD	<LOD	<LOD
	max	1.46	0.21	0.08	1.66
4	mean	0.02	<LOD	0.01	0.03
	SEM	0.01		0.01	0.02
	min	<LOD	<LOD	<LOD	<LOD
	max	0.15	<LOD	0.12	0.15
5	mean	0.19	0.03	0.01	0.23
	SEM	0.10	0.02	0.01	0.12
	min	<LOD	<LOD	<LOD	<LOD
	max	1.00	0.27	0.11	1.28
6	mean	0.15	0.03	0.16	0.35
	SEM	0.09	0.02	0.06	0.11
	min	<LOD	<LOD	<LOD	<LOD
	max	1.32	0.27	0.83	1.55
7	mean	0.30	0.07	0.29	0.66
	SEM	0.12	0.03	0.11	0.15
	min	<LOD	<LOD	<LOD	<LOD
	max	1.34	0.30	1.50	1.64
8	mean	0.22	0.05	0.23	0.50
	SEM	0.13	0.02	0.09	0.15
	min	<LOD	<LOD	<LOD	<LOD
	max	1.67	0.30	0.93	1.97

Only bees showed the presence of higher molecular weight PAHs such as BbF and BkF, whereas Ch was detected only in honey collected during September and October at Latium sampling stations.

All sampling sites showed the presence of PAHs. The lowest total PAH concentrations were detected in samples from sites 2−7 for the honeybees and honey, respectively, but no significant differences were detected between sites 1 and 8 and the other six sites. Furthermore, the mean PAH concentrations in honey samples were lower than those reported in honeybees, and no positive correlations were found between the compounds detected in bees and those in honey. This result highlights that contaminants can reach the honey by honeybees, but at the same time, the low concentrations and the different patterns found in fresh honey show that this matrix does not represent an appropriate bioindicator of environmental PAH contamination. We also tested, by a refractometer, the water content of honey samples and, probably, the presence in the majority of samples of a percentage of water higher than 18% led to lower PAH concentrations. In fact, fresh honey is a matrix with a high water content, and PAHs are hydrophobic compounds that tend to adsorb to lipidic matrices and not to polar matrices.

With regard to the monthly differences, great variability has been noted in the sampling sites. In honeybees the highest PAH concentrations were found in May for sampling sites 1−5 and 8. This contamination could be due to the use of smoke by beekeepers during the maintenance apiary works before the experimental protocol was begun. On the other hand, the samples collected at sites 6 and 7 showed the highest values in September. The honeybees from sites 2−8 showed the lowest PAH concentrations in October, whereas those from sampling station 1 showed the lowest concentrations in July. For May and July a statistically significant difference ($p < 0.01$) was reported only for sites 1−5. Honey showed the highest contamination values in July for all sites, whereas the lowest mean concentrations were found in June for samples in Abruzzi and in May and August for those in Latium. The season, weather, and botanical species are variables that should be considered when honeybees or beehive products are used as indicators because the different atmospheric conditions can modify the pollutant distribution. In fact, rain and

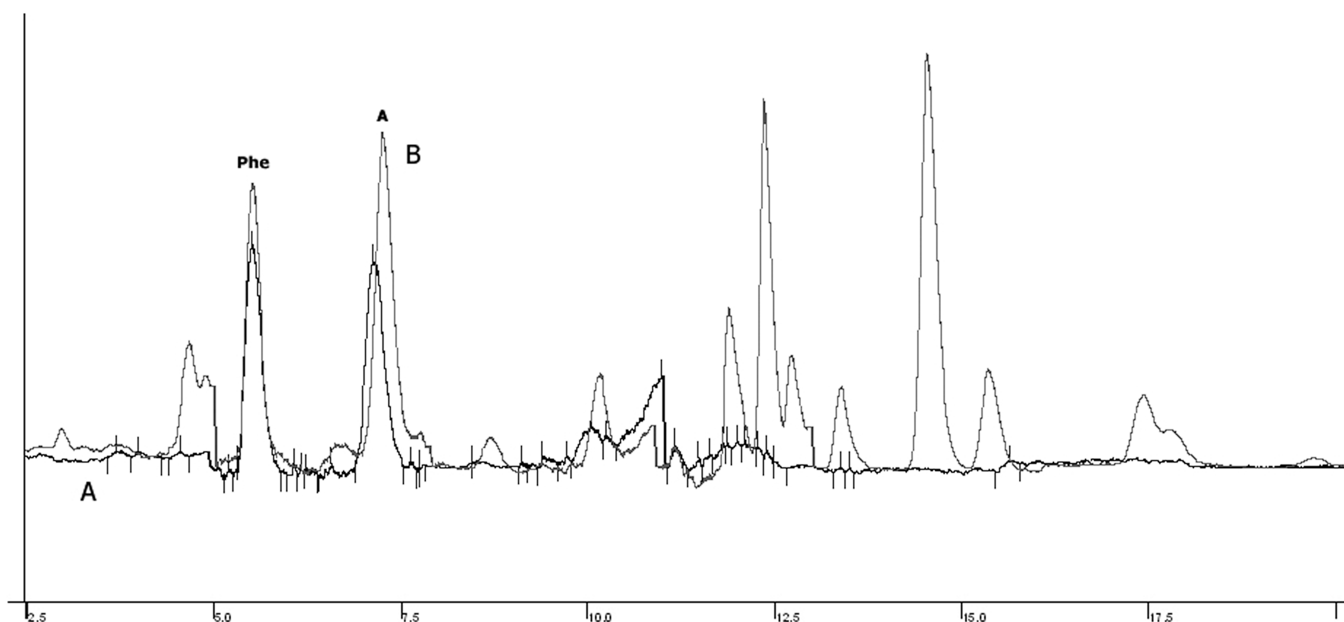

Figure 1. Chromatogram of honey sample (**A**) naturally contaminated and chromatogram of honey sample fortified with 10 ng mL^{-1} of PAHs mixture (**B**). Conditions: column, C18 Envirosep-pp; mobile phase, acetonitrile/water (65:35%, v/v) with gradient elution program; flow rate, 1.4 mL/min.

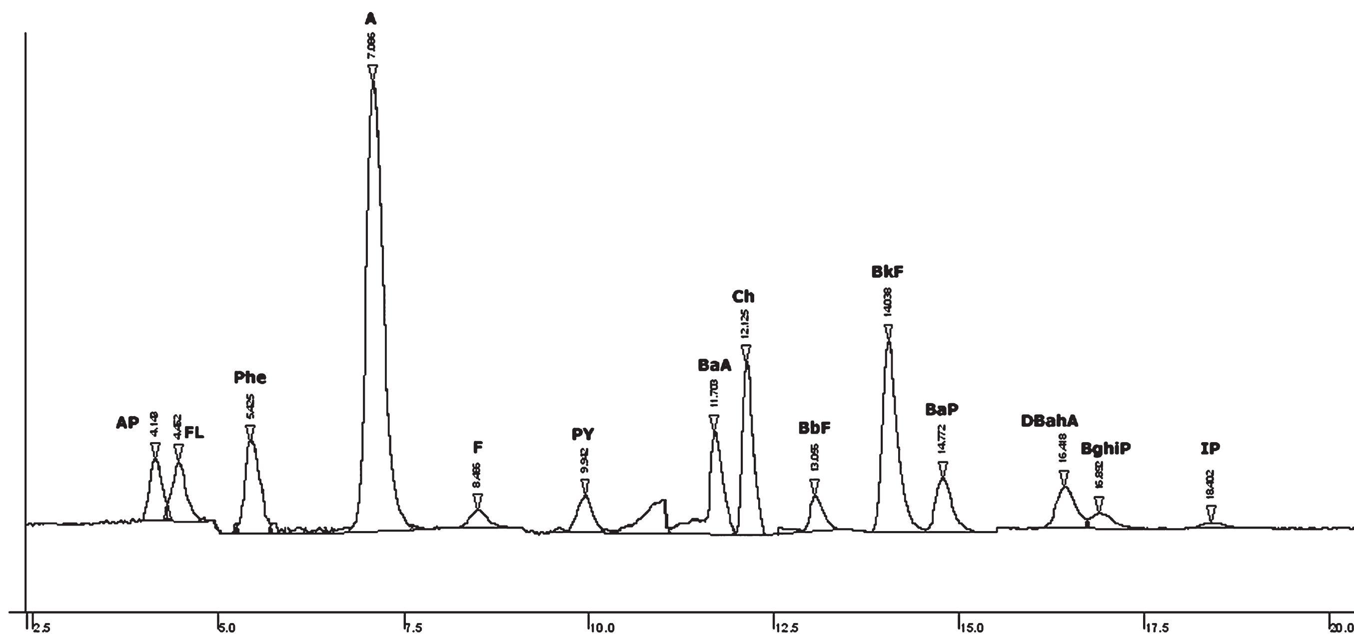

Figure 2. Chromatogram of PAH standards at 10 ng mL^{-1}. Conditions: column, C18 Envirosep-pp; mobile phase, acetonitrile/water (65:35%, v/v) with gradient elution program; flow rate, 1.4 mL/min.

Downloaded by monia perugini on August 26, 2009 | http://pubs.acs.org
Publication Date (Web): July 23, 2009 | doi: 10.1021/jf9011054

wind can clean the flowers and transfer the pollutants to other environmental sectors, and it is also important to consider that the nectar flow, which is usually greater in the spring than in the summer and autumn, could dilute the pollutants.

The honey contamination depends also on the botanical origin, and generally the nectar flowers with an open morphology and the honeydew are much more exposed to pollutants and thus more contaminated. In this study it was not possible to correlate the presence of specific PAH compounds with the botanical origin of the honey because the melissopalynological analyses showed that only four samples were monofloral honey, whereas the majority were wildflower honeys.

There are few reports of PAH pollution on bees, and the data obtained from this study cannot be compared with others carried out in the same area, but the use of honeybees as bioindicators can be considered to be an effective, cost-friendly, method for the investigation of the presence of organic contaminants in large areas. Furthermore, the lack of significant differences among the sampling stations and the PAH concentrations found in areas far from pollution sources, such as the natural reserves, strengthen the supposition that PAHs show a wide distribution in the environment and that for the airborne PAHs the most important transport medium is the atmosphere.

The results of this experimental study show the capacity of honeybees to reflect very low PAH concentrations also when the beehive was located far from any possible pollution source. These biological monitors provide an early warning of changing environmental conditions and could be an attractive way to assess anthropogenic changes over a long period, a signal for appropriate action to be taken. The use of honeybees as a continuous monitoring instrument could be well suited to address the terrestrial effects of human activity. Honeybees are more useful than honey in assessing the degree of PAH contamination in the environment.

ACKNOWLEDGMENT

We thank beekeepers Giuliano Cacciatore and Nino Mancini for excellent cooperation and helpfulness. We also thank Pacha Mama, CRA, and IZS for technical support.

LITERATURE CITED

(1) Raeymaekers, B. A prospective biomonitoring campaign with honey bees in a district of upper-Bavaria (Germany). *Environ. Monit. Assess.* **2006**, *116*, 233–243.
(2) Porrini, C.; Colombo, V.; Celli, G. The honey bee (*Apis mellifera* L.) as pesticide bioindicator. Evaluation of the degree of pollution by means of environmental hazard indexes. In *Proceedings of the XX International Congress of Entomology*, Firenze, Italy, 1996, Aug 25−31, 1996; p 444.
(3) Celli, G.; Maccagnani, B. Honey bees as bioindicators of environmental pollution. *Bull. Insectol.* **2003**, *56*, 137–139.
(4) Bromenshenk, J. J.; Carlson, S. R. Pollution monitoring of Puget Sound with honey bees. *Science* **1985**, *227*, 632–634.
(5) Porrini, C.; Sabatini, A. G.; Girotti, S.; Ghini, S.; Medrzycki, P.; Grillenzoni, F.; Bortolotti, L.; Gattavecchia, E.; Celli, G. Honey bees and bee products as monitors of the environmental contamination. *Apiacta* **2003**, *38*, 63–70.
(6) Crane, E. Bees, honey and pollen as indicators of metals in the environment. *Bee World* **1984**, *55*, 47–49.
(7) Przybylowski, P.; Wilczynska, A. Honey as an environmental marker. *Food Chem.* **2001**, *74*, 289–291.
(8) Bogdanov, S. Contaminants of bee products. *Apidologie* **2006**, *37*, 1–18.
(9) Hawkins, A. J. S.; Bayne, B. L. Physiological interrelations, and the regulation of production. In *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*; Gosling, E., Ed.; Elsevier: London, U.K., 1992; pp 171−222.
(10) Varanasi, U.; Stein, J. E.; Nishimoto, M. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*; Varanasi, U., Ed.; CRC Press: Boca Raton, FL, 1989; pp 94−149.
(11) Ramesh, A.; Walker, S. A.; Hood, D. B.; Guillen, M. D.; Schneider, K.; Weyand, E. H. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *Int. J. Toxicol.* **2004**, *23*, 301–333.
(12) ATSDR. *Toxicological Profile for Polycyclic Aromatic Hydrocarbons*; U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry: Atlanta, GA, 1995; pp 1−487.
(13) ATSDR. *Minimal Risk Levels for Hazardous Substances* (*MRLs*); Washington, DC, 1996; pp 1−347.

Received November 15, 2008. Revised manuscript received July 9, 2009. Accepted July 13, 2009.