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Using honey bee as bioindicator of chemicals in Campanian agroecosystems (South Italy)

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Abstract

Honey bees (*Apis mellifera* L.) have been used as biological indicators of Plant Protection Products (PPPs) in two intensely cultivated river areas of Campania Region (South Italy), Sarno and Volturno. Three areas were chosen for each site to place the monitoring stations, each consisting of two hives with colonies in good health, uniform strength and provided with "underbasket" cages for the collection of dead bees. All beehives were monitored continuously for the entire survey period (May to December 2006), also from the sanitary point of view. In addition to the counting of dead bees, we evaluated the strength of the family, the presence of diseases. The main crops in the plain of the Volturno were maize, wheat, orchard (mainly peach), tobacco, broad bean, clover, rape, cabbage and sugar beet. In the area of Sarno vegetables in both greenhouse and open field were predominant (melon, courgette, lettuce, salad rocket, green bean, onion, sweet pepper, chili pepper, turnip, tomato and fennel), followed by fruit (persimmon and hazelnut). Woodlands and extensive urbanized areas were also surveyed. The critical threshold of mortality (250 dead bees per station per week), was totally exceeded for 53 times and 40 samples were subjected to chemical and palynological analysis: 18 coming from the Volturno area and 22 from the Sarno. Overall, 80% of these (32) were positive to the presence of at least one pesticide residue. The active ingredients found were all organophosphorus, except in one case (cypermethrin), and were detected mainly between May and July in the plain of the Volturno (Caserta) and in the period from July to September in three stations of Sarno (Salerno). The chlorpyrifos-ethyl was the active substance most frequently found in 40 analyzed samples (32% of residues), followed by fenitrothion (20.7%), pirimiphos-methyl (18.8%) and dimethoate (11.3%). Surveys and analysis carried out, including those palynological, show that most of the mortalities were caused by the improper use of PPPs such erroneous treatments during flowering, the non-mowing of the native flora and the treatment performed in the presence of wind with a resulting drift effect. One sample of bees, obtained by mixing the bees collected in three stations of Volturno and one sample of wax from one station of Volturno (Cancello ed Arnone), were analyzed during the investigation, to detect the presence of dioxins. While in the bee sample the level of dioxins was below the detection limit, in the wax sample a 2.55 mg I-TEF/Kg $\times 10^{-6}$ residue was found. Since no reference values for the wax exist, in order to interpret this result we can consider that in groundwater the maximum acceptable value is 4.0 $\times 10^{-6}$, while in animal fat, as well as in milk and dairy, is 2.0 $\times 10^{-6}$ mg I-TEF/Kg.

Key words: *Apis mellifera*, biomonitoring, pollution, pesticides, dioxins.

Introduction

The pesticide pollution in intensively cultivated areas represents a dangerous phenomenon because these products accumulate in vegetation, water and soil and cause damages to beneficial organisms such as honey bees (*Apis mellifera* L.) (Porrini *et al*., 2002).

In organic and integrated agricultural areas, as well as in typical production areas, the control of this type of pollution is carried out through the chemical analysis of environmental matrices. Moreover, the use of organisms as biological indicators for the evaluation of this kind of pollution is widely accepted (Balayiannis and Balayiannis, 2008; Zhelyazkova, 2012).

The bioindicator is a sentinel used as interpreter of complex conditions (Schmidt di Friedberg, 1986). The information it gives enclose the interaction of many factors, often difficult to measure directly. It is therefore useful to detect the presence and to report the dynamics of processes whose measure would be otherwise time and money consuming. The requirements of a good bioindicator may be different depending on the nature of the bioindicator, the type of response that is able to express, the type and duration of the environmental alteration to be detected (Sartori, 1998). Various attributes make honey bees a good bioindicator: first, it is an almost ubiquitarian insect; then, it is easy to rear and it enters in contact with substances present in the environment since it has an intense foraging activity; each beehive has thousands foraging bees, renewing periodically, that can be considered as thousands detectors (Celli, 1983; Celli and Porrini, 1991; Accorti *et al*., 1991; Pinzauti *et al*., 1991; Celli and Maccagnani, 2003). The mean flight radius of a forager bee is about 1.5 km, so that the foraging area of a colony is about 7 km^2 (Crane, 1984). In this case, the honey bee shows a high sensitivity towards the most widely and intensely used pesticides in both urban and agricultural ecosystems.

Our investigation was carried out to monitoring the pesticide pollution in two intensively cultivated and fertile areas of the Campania Region (South Italy) near two rivers of the region, Sarno and Volturno. In addition, the analysis of dioxins, an important pollutant already detected in the surveyed area in other environmental matrices including soil, sediment and air (Menegozzo *et al*., 2008), was also carried out in samples of dead bees. The aim of dioxin analysis was to assess if the honey bee was able to reveal its presence in an area, such as the plain of the Volturno, where these substances were found both in environmental and food matrices, in previous years. Whereas dioxins bind easily to fat, due to it is lipophil propriety, also the wax of the hive was analyzed.

Materials and methods

Monitoring stations

The monitoring stations, consisting of two beehives (A and B), Dadant-blatt type with 10 frames, homogeneous in colony strength, were located in two areas in Campania region, Sarno and Volturno plains. These two areas were different for the agricultural pattern but similar in orography and climate.

Three locality were chosen within each area (figure 1) and for each locality were installed one monitoring station:

- Area Volturno River: Capua (VOL1), Castelvolturno, locality Torre di Pescopagano (VOL2), Cancello ed Arnone (VOL3);
- Area Sarno River: Nocera Superiore (SAR1), San Marzano sul Sarno (SAR2) and Scafati (SAR3).

The VOL1 station was placed near one non-migratory apiary of 50 hives located within a citrus orchard and *Eucalyptus*, whereas the station VOL2 was placed near a hundred hives apiary, next to a river basin with presence of bamboo and *Eucalyptus*. VOL3 was set up beside a 100 hives apiary, located in a livestock farm where mainly Brassicaceae, *Eucalyptus* and maize were cultivated.

SAR1 station was placed into a 100 hives standing apiary located within an orchard with cherries, citrus and persimmon trees, while SAR2 was near a persimmon orchard and a horticultural area. The SAR3 hives were placed 20 m from Sarno River, within horticultural crops as tomatoes, lettuce and salad rocket. SAR2 and SAR3 hives were placed just for the time of the investigation.

All the beehives were provided with cages for dead bees sampling (underbasket type) and were constantly monitored, from a sanitary point of view also.

Work plan

From May to December 2006 the following detections were performed every 18 days in each monitoring station:

- Count of dead and dying bees in the underbasket cages. When the critical threshold of mortality was exceeded, the dead bees were sampled for analysis. Critical mortality threshold was defined in 18 dead bees/hive/day (250 dead bees per station per week) (Porrini *et al*., 2002; Porrini *et al*., 2003);
- Colony strength control through direct observation and pictures of the honeycombs; pathologies evaluation through observation of symptoms.

Information has been collected regarding beekeeping management of the apiaries and the most frequent pathologies.

Weather data, relative to the investigation period, were provided by ARPA (regional agency for the environment) of Campania.

Vegetation detections

In the two areas, surveys were carried out with the aid of crop maps and satellite photos for the determination of plant species in the examined zone, with particular reference to the foraging area of the honey bees. Then, collected data were cross-checked with those resulting from palynological analysis.

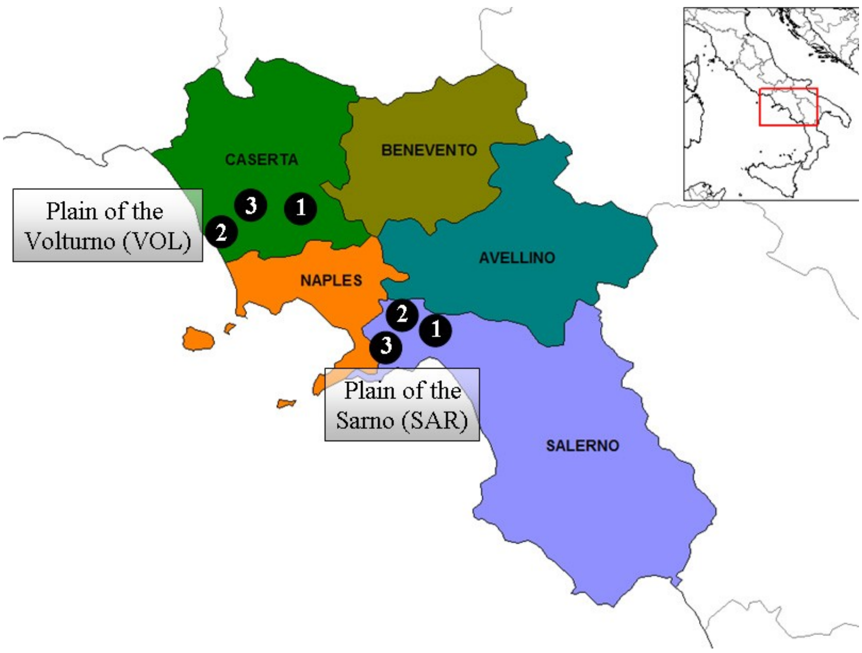

Figure 1. Monitoring stations in the Campania Region (Area Volturno River: Capua VOL1, Castelvolturno, locality Torre di Pescopagano VOL2, Cancello ed Arnone VOL3. Area Sarno River: Nocera Superiore SAR1, San Marzano sul Sarno SAR2 and Scafati SAR3).

Sampling preparation

During the survey, 40 samples of dead bees were collected from the underbasket cages. They were taken to laboratories, cleaned up from foreign bodies, labelled and frozen at −20 °C until chemical and palynological analysis were carried on. We paid particular attention to the temperature monitoring during all the steps of the preparing process and transport of the samples.

Chemical analysis

Each sample was vacuum freeze-dried and pulverized at the laboratories of CRA-API (Bologna). Afterwards, an amount of 10 g of the resulting bee powder was treated with a mixture of cicloesane/dicloroetane in order to extract any possible analytes.

After filtration through a fiberglass screen, the extract was subjected to gel permeation chromatography (stationary phase BioBeads SX3 200-400 gelled mesh, mobile phase dichloromethane-cyclohexane 50:50). The eluate was concentrated, taken up in n-hexane and placed in sealed vials. Subsequently, the purified extract was analyzed by either gas chromatography capillary column (Supelco SPB608) and nitrogen-phosphorus detector (NPD) or gas chromatography - mass spectrometry with capillary column J & W DB-35ms. Tests were also conducted in the laboratory of CSA (Centro Studi Ambientali) in Rimini.

For neonicotinoids detection (imidacloprid and thiamethoxam) 3.5 g from each sample was used. After the extraction, the eluate was loaded on the HPLC with coulometer detector (Rancan *et al.*, 2006). Residues of pesticides in the sample were expressed as mg/kg (ppm).

Palynological analysis

Pollen washed off about 30 honey bees collected in the underbasket for each sample was analyzed in the laboratory of the Department of Botany AR.BO.PAVE - University of Napoli "Federico II" and in the palynological laboratory in CRA-API (Bologna), in order to determine pollens and to clarify, with the help of vegetation maps, which path the pollen followed and which species had been visited by foragers bees. This kind of method was particularly useful in order to detect whether crops had been contaminated by pesticides. Regarding laboratory methodologies we referred to Louveaux *et al.*, 1978.

Index of Environmental Hazard assessment

Index of Environmental Hazard (IEH) allows to categorize the chemical hazard level of a territory. This index takes into account monthly mortality of honey bees and some parameters of pesticides found in dead bees as their environmental persistence and toxicity (Porrini *et al.*, 2002).

Dioxin analysis

By mean of the bioaccumulation phenomenon, dioxins can concentrate through the food chain. For economical reasons, only one sample from Volturno area, obtained from honey bees of the three monitoring stations, was analyzed with respect to dioxins; one more wax sample was collected in VOL3. Analyses were performed by CSA in Rimini, according to the official EPA method 1613 (EPA, 1994), using high resolution GC (HRGC) coupled with high-resolution MS (HRMS) specific for dioxins and furans (PCDD and PCDF).

Results

Vegetation detections

In Capua (VOL1) a prevalence of maize (24%) and wheat (16%) with a patchy distribution was observed, whereas the orchards (mainly peach, 12%) were located close to Volturno River, south of the surveyed area. The 6% of the area was represented by tobacco, mainly grown in greenhouses, and sugar beet.

Cancello ed Arnone area (VOL3) had the same crop composition; maize was the most significant crop (35%), tobacco area was increased (14%) and orchards was less spread and located at the borders of the experimental area.

On the contrary, in Castel Volturno area (VOL2), maize (6%) and wheat (5%) were replaced by vegetables such as broad bean, clover, rape and cabbage (29%). This area, located west near the sea, was characterized by seashore and maritime vegetations.

In Sarno River area, the monitoring stations of San Marzano sul Sarno (SAR2) and Scafati (SAR3) were predominant cultivated by vegetable crops, both in greenhouse and open field (70% and 75% respectively). There were different harvesting periods, with melon, courgette and lettuce in the first season and salad rocket, green bean, onion, sweet pepper, chili pepper, turnip, tomatoes and fennel subsequently. The presence of orchards (about 10%), mainly persimmons and hazelnuts, was significant.

At Nocera Superiore (SAR1), located in a foothill area, many orchards were grown, persimmon in particular (42%). It is crossed, from South-West to North-East, by urban settlements and anthropic areas (28%) and east of that, it has a wide woodland zone. Horticultural and forage crops take up less than 10% of the entire surface and are located next to the urban area.

Pesticide application operation

The information about Plant Protection Products (PPPs) used in Sarno area (in Volturno area a similar dataset was not available) during the investigation period were provided by Plant Protection Service of the Campania Region.

Among employed products, 10 organic nitrogen compounds, both acaricides and fungicides were found; all of them are irritant, slightly or moderately toxic to human and slightly or moderately toxic to honey bees, with some exceptions which result highly toxic. Some organophosphorus and carbamate compounds used, were harmful for human and highly toxic for honey bees (PPDB, 2007).

Meteorological data

For Volturno area we report the data provided by the ARPA meteorological station of Vitulazio (CE), North of Capua, while for Sarno area, the ARPA meteorological station of Ercolano (NA) has been used, since it is the closest to the three experimental stations (about 30 km far).

The data show that in Volturno area, the maximum temperature exceeded frequently 30 °C, while in Sarno area that situation rarely occurred.

A similar number of rainfall days were recorded in both experimental areas (45 days in Sarno and 44 days in Volturno), but Sarno had the highest amount of precipitations.

Volturno area is more arid, and this is evident also considering that 320 mm of rain fell between May and October, but 80 of them were recorded in a single day.

Bee mortality and colony strength

Figure 1 shows the localization of the six monitoring stations. Between May and June, in the apiaries of VOL3, SAR1 and SAR2, some peaks of mortality were observed.

All the hives maintained constant both the number of honey bees and the brood extension for the time of the survey, except for SAR2 station, where a strong decline of honey bees and brood was observed from the mid-June to the mid-September. Overall the critical threshold of mortality was exceeded for 53 times.

Chemical analysis

A total of 18 samples from the area Volturno and 22 samples from Sarno were analyzed. In the Volturno area, 16 of the overall samples were positive (89%) and in the Sarno area, 16 samples (73%) contained at least one pesticide (tables 1, 2 and 3).

Palynological analysis

From May to September when the mortality threshold was overcome the pollen collected in the dead honey bee bodies were analyzed (table 4).

In Volturno area the pollen identification revealed a constant presence of *Eucalyptus* in high quantities and traces of Graminaceae and *Sambucus* in the first period.

Table 1. Results of the dead bees samples analysis in the Volturno River zones. IEH: Index of Environmental Hazard (A_1: persistent, A_2: worrying, A_3: substantial, A_4:considerable, B_1: high elevated, B_2: important, B_3: widespread, C_1: medium average, C_2: medium-low, C_3: moderate, D_1: low, D_2: limited, D_3: minimal, D_4: absent).

Station	Period	Monthly mean of weekly mortality	N. of samples	Active ingredients	Amount (mg/kg)	IEH
VOL1 Capua	May	88	0			D4
	June	811	0			C1
	July	53	1	Chlorpyrifos-ethyl	0.045	C1
	August	29	0			D4
	September	271	1	Pirimiphos-methyl	0.005	C3
	October	0	0			D4
	November	0	0			D4
VOL2 Castelvolturno, Torre di Pescopagano	May	289	1	Chlorpyrifos-ethyl	0.173	C1
	June	351	1	Dimethoate Parathion-ethyl Diazinon Chlorpyrifos-ethyl	0.010 0.006 0.046 0.201	C2
	July	171	2	Fenitrothion Chlorpyrifos-ethyl Chlorpyrifos-methyl Chlorpyrifos-methyl	0.192 0.192 0.022 0.492	D1
	August	0	0			D4
	September	99	0			D4
	October	22	0			D4
	November	0	0			D4
VOL3 Cancello ed Arnone	May	1079	2	Fenitrothion Fenitrothion Chlorpyrifos-ethyl Chlorpyrifos-ethyl	1.857 0.880 0.097 0.220	A3
	June	1498	2	Fenitrothion Chlorpyrifos-ethyl Chlorpyrifos-ethyl	0.139 0.139 0.138	A2
	July	709	4	Fenitrothion Fenitrothion Fenitrothion Chlorpyrifos-ethyl Chlorpyrifos-ethyl	0.066 0.099 0.263 0.007 0.019	B2
	August	932	4	Chlorpyrifos-ethyl Fenitrothion	0.031 0.094	A4
	September	284	0			D2
	October	20	0			D4
	November	0	0			D4

Table 2. Results of the dead bees samples analysis in the Sarno River zones. IEH: Index of Environmental Hazard (A_1: persistent, A_2: worrying, A_3: substantial, A_4:considerable, B_1: high elevated, B_2: important, B_3: widespread, C_1: medium average, C_2: medium-low, C_3: moderate, D_1: low, D_2: limited, D_3: minimal, D_4: absent).

Station	Period	Monthly mean of weekly mortality	N. of samples	Active ingredients	Amount (mg/kg)	IEH
SAR 1 Nocera Superiore	May	140	0			D4
	June	140	0			D4
	July	348	1	-		D2
	August	309	4	Malathion Pirimiphos-methyl Pirimiphos-methyl Dimethoate Dimethoate Chlorpyrifos-ethyl Chlorpyrifos-ethyl Cypermethrin Pirimiphos-methyl	0.074 0.014 0.010 0.037 0.040 0.006 0.030 26.855 0.010	C2
	September	141	0			D4
	October	32	0			D4
	November	0	0			D4
SAR 2 San Marzano sul Sarno	May	1158	2	Chlorpyrifos-ethyl	0.023	B1
	June	4607	1	-		C1
	July	1123	4	Omethoate Pirimiphos-methyl Pirimiphos-methyl Pirimiphos-methyl	0.063 0.021 0.021 0.042	B1
	August	856	4	Pirimiphos-methyl Pirimiphos-methyl Dimethoate Chlorpyrifos-ethyl	0.014 0.010 0.010 0.058	B1
	September	941	2	Fenitrothion Pirimiphos-methyl Fenitrothion	0.022 0.040 6.999	B1
	October	305	2	Chlorpyrifos-ethyl Fenitrothion	0.061 0.399	C2
	November	49	0			D4
SAR 3 Scafati	May	0	0			D4
	June	118	0			D4
	July	26	1	Dimethoate Chlorpyrifos-methyl Parathion-methyl Pirimiphos-methyl Chlorpyrifos-ethyl	0.010 0.005 0.001 0.001 0.002	D1
	August	19	0			D4
	September	181	1	Dimethoate	0.010	D1
	October	0	0			D4
	November	0	0			D4

Table 3. Active ingredients found in the samples of dead bees.

Pesticide	Pesticide class	Purpose of use	LOQ ($\mu g\ kg^{-1}$)	LOD ($\mu g\ kg^{-1}$)	Number of positive samples	Frequency (%)	Average concentration (mg/kg)
Chlorpyrifos-ethyl	Organophosphate	I	10	1	16	40	0.092
Chlorpyrifos-methyl	Organophosphate	I + A	10	1	3	7.5	0.173
Dimethoate	Organophosphate	I	100	10	6	15	0.016
Fenitrothion	Organophosphate	I	10	1	11	27.5	0.971
Parathion-methyl	Organophosphate	I	10	1	1	2.5	0.001
Pirimiphos-methyl	Organophosphate	I	10	1	10	25	0.015
Omethoate	Organophosphate	I + A	50	10	1	2.5	0.063
Cypermethrin	Pyrethroid	I	100	10	1	2.5	26.855
Malathion	Organophosphate	I	10	1	1	2.5	0.074
Parathion-ethyl	Organophosphate	I + A	10	1	1	2.5	0.006
Diazinon	Organophosphate	I	10	1	1	2.5	0.046

A: Acaricide; I: Insecticide; LOQ: limits of quantification; LOD: limit of detection.

Table 4. Pollen found on the bodies of dead bees analyzed for pesticides detection. 1 *Acacia*; 2 *Acer*; 3 *Ailanthus*; 4 *Allium* form; 5 *Ambrosia*; 6 Anacardiaceae; 7 *Artemisia*; 8 *Borago*; 9 *Brassica* form; 10 *Bryonia*; 11 *Calystegia*; 12 *Carex*; 13 *Castanea*; 14 *Cerinthe*; 15 Chenopodiacee/Amaranthacee; 16 Chenopodiaceae; 17 Cistaceae; 18 *Citrullus*; 19 *Citrus*; 20 *Clematis*; 21 Compositae; 22 Compositae A; 23 Compositae H; 24 Compositae J; 25 Compositae S; 26 Compositae T; 27 *Convolvulus*; 28 Cruciferae; 29 Cucurbitaceae; 30 Cupressaceae; 31 *Datura*; 32 Dipsacaceae; 33 *Echium*; 34 *Eucalyptus* form; 35 *Euphorbia*; 36 *Galega*; 37 Graminaceae; 38 *Hedera*; 39 *Hedysarum*; 40 Labiatae; 41 *Lagerstroemia*; 42 Liliaceae; 43 *Lotus*; 44 *Magnolia*; 45 Malvaceae; 46 *Medicago*; 47 *Mercurialis*; 48 *Ocimum*; 49 *Olea*; 50 Oleaceae; 51 *Ononis*; 52 Palmae; 53 *Papaver*; 54 *Parietaria*; 55 *Parthenocissus*; 56 Pinaceae; 57 *Pinus*; 58 *Plantago*; 59 *Polygonum persicaria* group; 60 *Prunus* form; 61 *Pyrus* form; 62 *Quercus ilex*; 63 *Quercus robur* group; 64 *Reseda*; 65 *Robinia*; 66 *Rosa* form; 67 *Rubus* form; 68 *Rumex*; 69 *Sambucus nigra*; 70 *Sambucus ebulus*; 71 Solanaceae; 72 *Trifolium incarnatum*; 73 *Trifolium pratense* group; 74 *Trifolium repens* group; 75 Umbelliferae; 76 Urticaceae; 77 *Verbascum* form; 78 *Viburnum*; 79 *Zea*.

2006	VOL1		VOL2		VOL3		SAR1		SAR2		SAR3	
date	Hive A	Hive B	Hive A	Hive B	Hive A	Hive B	Hive A	Hive B	Hive A	Hive B	Hive A	Hive B
24 V			8, 13, 19, 22, 25, 28+, 30, 34++, 37+, 39, 43, 51, 52, 53, 56, 58, 62, 63, 68, 76, 78									
17 VI	34, 37, 54, 67, 69	34, 35, 37, 39, 49, 53, 54, 66, 71	9, 25, 34, 35, 49, 53	10, 25, 28, 33, 34, 37, 49, 50, 54, 55, 71, 74		8, 21, 34, 37, 49, 53, 54, 57, 69, 71, 73			9, 13, 26, 29, 65, 71	13, 69, 71, 73		
3 VII				12, 22, 25, 32, 34, 37, 55, 56, 58, 70, 74	5, 13, 16, 18, 22, 24, 25, 26, 27, 28, 29, 30, 33, 34+++, 36, 37+, 38, 39, 40, 43+, 44, 45, 46, 47, 53, 56, 58, 67, 70+, 73+, 74, 76						1, 4, 13, 16, 17, 19, 22, 26, 27, 28+, 33, 34+, 37, 40, 44, 47, 50, 55, 56, 61, 62, 63, 67, 74, 76, 77	
10 VIII						5, 7, 16, 18, 20, 23, 26, 27, 28, 32, 34+, 37, 40+, 42, 43, 46, 58, 67, 70++, 74, 75, 79			13, 23, 28, 33, 34, 37, 42, 52, 56, 75, 76			
29 VIII					7, 13, 19, 25, 26, 34, 37, 70, 78, 79		7++, 11, 13, 16, 23, 25, 26, 27, 28+, 34, 40, 41, 47, 48, 52, 53, 56, 58, 67, 71, 74, 75	7, 11, 13, 16, 23, 25, 26, 27, 28++, 34, 40, 41, 47, 48, 52, 53, 56, 58, 67, 71, 74+, 75	7++, 11, 13, 16, 23, 25, 26, 27, 28+, 34, 40, 41, 47, 48, 52+++, 53, 56, 58, 67, 71, 74, 75			
16 IX		7+, 8, 16, 23, 24, 28+, 31, 32, 34, 38, 58, 59, 72, 75							7, 13, 16, 28, 34, 38, 47, 48, 52+, 75	7, 13++, 28, 34, 42, 52+, 55, 75,	7++, 13, 16, 23, 28, 34, 42, 48, 52++, 75	
3 X	19, 34, 54	13, 34, 54, 71	9, 13, 14, 15, 34, 37, 53, 54		9, 34, 37, 53, 71, 73	13, 34, 64, 71	2, 9, 13, 54, 71		2, 13, 34, 53, 71	3, 13, 21, 53, 71		
19 X									6+, 7+, 13, 16, 19, 23, 26, 28, 31+, 34, 38, 40, 42, 45, 47, 48, 52+, 56, 58, 59, 60, 67, 71, 74, 75			

Pollen: +++ = most abundant; ++ = abundant; + = present; number alone = traces.

During the whole season it has been seen low presence of agricultural crops pollen.

Palmae and Pinaceae pollen were peculiar of the coastal area of Castelvolturno (VOL2). Pollen analysis from Nocera Superiore (SAR1) samples, collected in August, shows the strong influence of woodlands (*Castanea*, *Eucalyptus*, *Pinacea).*

At the end of August, pollen from Graminaceae and *Artemisia* was predominant; furthermore, pollen loads from wild plants as Chenopodiaceae, *Poligonum*, *Trifolium*, Umbelliferae and ornamental species as *Clematis*, *Echium*, Liliaceae and *Verbena* were detected.

Pollen analysis relating to San Marzano sul Sarno (SAR2), shows that honey bees have visited public gardens (*Lagerstroemia*, Palmae, *Parthenocissus*, *Echium*, *Lilium*), home gardens (*Labiateae*, *Ocimum* and Solanaceae), tree rows (*Eucalyptus*, Pinaceae) and wild species (Compositae, *Convolvulus*, Chenopodiaceae, *Trifolium*).

The pollen spectrum of Scafati (SAR3) is similar to the former (SAR2) with the additional presence of *Citrus*, *Allium* and Cucurbitaceae pollen and tree species as *Quercus*, *Acacia* and *Magnolia*.

IEH valuation

In Volturno area, IEH highlighted (table 1):

- 57.1% of values with low environmental hazard (D);
- 23.8% of values with intermediate environmental hazard (C);
- 4.8% of values with high environmental hazard (B);
- 14.3% of values with maximum environmental hazard (A).

For Sarno area, IEH indicates: (table 2):

- 66.7% of values with low environmental hazard (D);
- 14.3% of values with intermediate environmental hazard (C);
- 19.0% of values with high environmental hazard (B).

In general, during the spring-summer season IEH values were higher; the stations with the most concerning IEH values was Cancello ed Arnone (VOL3) and San Marzano sul Sarno (SAR2).

Considerations on the monitoring of pesticides in various areas

Capua (VOL1)

During two visits on June 17th and July 3rd, we observed high mortality, far beyond the natural threshold, with 58 and 39 dead bees/day/hive respectively.

In the dead bee samples collected in the hive A on July 3rd, chlorpyrifos-ethyl residues have been detected; this product is widely used in the area, against various phytophagous in orchards (peach, apple and cherry orchards).

A second peak of mortality has been registered on September 16th in the hive B with a mean mortality of 32 dead bees/day/hive. In the sample we detected residues of pirimiphos-methyl, used in horticulture and in Pomaceae and Drupaceae orchards.

Wild species pollen found upon dead bee bodies points out that honey bees foraged in an orchard area next to Volturno River. In all the other visits we didn't observe significant mortalities and the colonies developed well.

The IEH index shows a slight environmental hazard in June, July and September and a low hazard in the other periods (table 1).

Castelvolturno (Torre di Pescopagano) (VOL2)

The first mortality peak was observed on June 17th with a mean of 335 dead bees/day/hive. Chemical analysis of dead bee samples revealed residues of dimethoate, parathion-ethyl, diazinon and chlorpyrifos-ethyl, all of them being extremely toxic for honey bees. Dimethoate, chlorpyrifos-ethyl and diazinon are mainly employed in orchards, while parathion-ethyl is used almost on every crop, although the guidelines don't provide for its use.

The pollen found on dead bee bodies doesn't suggest specific foraging areas, but orchards are present only along Volturno River shores, South respect to the monitored area.

The mortality exceeded the critical threshold in hive A on May 24th and July 3rd (27 and 33 dead bees/day/hive respectively); in the first date, residues of chlorpyrifos-ethyl were found, while in the second date samples we detected also chlorpyrifos-methyl and fenitrothion.

All these active ingredients are employed in orchards in both periods. This might suggest us that honey bees foraged in the same zone indentified for the mortality peak of June 17th.

Afterwards the mortality remained below the threshold and no pesticide residues were found. In May and June the IEH index was medium, low in July and absent (D4) in the remaining period (table 1).

Cancello ed Arnone (VOL3)

The number of dead bees remained high until the 30th of August. In particular, in both hives we registered mortality peaks on May 24th, July 3rd and August 29th with 100, 160 and 80 dead bees/day/hive respectively. In all cases residues of chlorpyrifos-ethyl were detected while in the first two dates also fenitrothion was found. The wide maize area could explain the use of chlorpyrifos-ethyl against the European corn borer, even if this product's employment is not allowed by regional production's technical policy.

Another possible employment of this active ingredient, permitted by the production regulations, is against click beetles (Elateridae) on tobacco.

The analysis of the pollen found on dead bees bodies of hive A, on July 3rd proves the presence of *Citrullus* and Cucurbitaceae (table 4); these crops might have been contaminated through treatment drift in orchards. These zones are limited to the North-West area. The IEH index shows high values from May to August, modest values in September, and goes down to zero in the remaining period (table 1).

Nocera Superiore (SAR1)

Between July 20th and August 29th a high mortality, slightly higher than the natural threshold (23 and 27 dead bee/day/hive respectively), has been registered in both hives.

August samples (on 10th and 29th) resulted positive to the chemical analysis. In the first date pirimiphos-methyl and malathion were detected; the latter was used, according to Campania production policies, in persimmon and citrus orchard against the Medfly, *Ceratitis capitata* (Wiedemann). These crops are cultivated near the hive, and in the whole zone with a patchy distribution. Supposedly pirimiphos-methyl, was used on olives, although it is not planned in the production policies.

The biggest olive grove in the area is located west of the experimental hive and a smaller one is South-East. Pollen spectrum (*Castanea*, Pinaceae) indicates a foraging activity on the woodland areas near this second olive grove. Residues of pirimiphos-methyl, chlorpyrifos-ethyl and dimethoate, in association with a remarkable amount of Cruciferae and wild species pollen has been detected in honey bees collected on August 29th (table 4). This suggests that honey bees visited the turf beneath and surrounding the peach orchards treated with chlorpyrifos-ethyl against *Grapholita molesta* (Busck) and *Anarsia lineatella* Zeller and the olive groves treated against the olive fly, *Bactrocera oleae* (Rossi). This crop association traces back to an area West and on other area South-East of the monitoring station. In the honey bees collected in hive A on August 29th, a very high concentration of cypermethrin (26.855 ppm) was found. This broad spectrum pyrethroid is characterized by a rapid and lasting effectiveness. It is used on a large amount of crops, from orchard to horticultural crops and from tobacco to maize. Cruciferae pollen predominance on honey bee bodies indicates a possible cypermethrin use either on cabbages (even if it's not much present in the area, less than 5%) or on olive and abandoned orchards near the station.

On October 3rd the registered mortality was 20 dead bees/day/hive, and no residue was found.

During August the IEH index resulted medium; limited or absent in the remaining period (table 2).

San Marzano sul Sarno (SAR2)

Honey bees mortality was far beyond the critical threshold for the whole investigation period. On June 17th the number of dead bees was surprisingly high: 11,063 in the hive A and 5,000 in the hive B, with an average number of 432 dead bees/day/hive. As a consequence the colonies were strongly weakened; while the hive A presented low strength until the end of the surveys, the hive B was declared dead on October 3rd.

Unexpectedly the chemical analysis has revealed no active ingredient. In the same sample, also analyzed at CSA laboratories in Rimini, even with a broader range of the sought active ingredients, no residue was found.

Three different hypotheses were formulated:

1. The mortality was detected soon after a rainy period and with maximum temperatures below 15 °C. Therefore it could be hypothesized that the residues of one or more chemical treatments was washed out by the rainfalls;
2. The mortality was caused by an agrochemical different from all of the sought active ingredients;
3. The proximity of the station to the Sarno River might have induced honey bee to supply with that water, becoming the victims of a poisoning due to the pouring of some extremely toxic pollutant (Menegozzo *et al*., 2008).

The mortality stayed very high even in the following sampling (207 dead bees/day/hive on July 3rd). In that date omethoate and pirimiphos-methyl traces were found. The latter was constantly detected in all the next dead bees samples analyzed until September 16th, with mean daily mortalities per hive fluctuating between 89 and 33 honey bees (maximum and minimum values found on July 20th).

Pirimiphos-methyl employment was used against various phytophagous of horticultural plants, abundant in the area, although it was not planned in the regional disciplinary of IPM in Campania.

In that period the omethoate use is scheduled on cherry trees, within mixed orchards east of the monitored area. No palynological analysis is available. Residues of chlorpyrifos-ethyl were found, in association with Cruciferae pollen (table 4) and with at least another agrochemical on August 10th and on September 16th, while on May 29th and on October 3rd, it was found alone. In these last two cases the mortalities was 49 and 83 dead bees/day/hive respectively.

The use of that active ingredient against *Cydia pomonella* (L.) is supposable. The correspondent pollen analysis shows the concurrent presence of pollen from wild, horticultural and ornamental plants; this suggests that honey bees might have gone in orchards next to the urban centre located right in the middle of the surveyed area. In the latter case (on October 3rd) it is possible to hypothesize a chlorpyrifos-ethyl use on horticultural species. On October 3rd, the hive B has showed a nearly null activity and the colony was declared dead. June poisoning had presumably impaired the colony development. In this station fenitrothion was found on September 16th (hive B) and on October 19th (hive A) with a mortality level of 48 and 36 dead honey bee/day/hive respectively. In the sample collected on September 16th was detected pollen from Umbelliferae and Cruciferae as well as *Parthenocissus* and Palmae (table 4); that might indicate the use of the product in an area next to the urban centre with wild species and/or a turnip field, even if the use of fenitrothion can be considered ubiquitarian in all the monitored area. Anyhow, in both colonies, a decline of worker bees and brood was observed, that means the local honey bees were subject to a strong stress.

August 10th we noticed, hive B had no more brood.

In this area we registered an IEH fluctuating between medium and high level for the whole period (table 2).

Scafati (SAR3)

In two occasions, on July 3rd and on September 16th residues of dimethoate were found on dead honey bees, although the mortality rate was below the natural value.

This active ingredient, according to the disciplinarians, is employed against *Rhagoletis cerasi* (L.). Traces of chlorpyrifos-ethyl, chlorpyrifos-methyl, pirimiphos-methyl and parathion-methyl were detected in the sample of July 3rd. Chlorpyrifos-methyl can be used both in orchards and in horticultural species since it has a broad spectrum activity.

Table 5. Analysis of dioxins.

Matrix	Honey bees	Wax
Date	February 8, 2007	March 2, 2007
Parameter	Sum of PCDD, PCDF (TEF conversion)	Sum of PCDD, PCDF (TEF conversion)
Result	< 0.000001	0.00000255
Unit of measure	mg I-TEF/Kg	mg I-TEF/Kg
Detection limit	1.00E-06	1.00E-06
Method	EPA 1613B 1994	EPA 1613B 1994

The same applies to the other two active ingredients, although they were not scheduled in the regional production policies.

The presence of pollen from Cruciferae and *Allium* on the dead bee bodies indicates that treatments have been carried out on horticultural plants (table 4). The IEH hazard level indicating different classes of pollution, results low in July and September, and absent in the remaining period (table 2).

Dioxin analysis

The analysis of a wax sample coming from Cancello ed Arnone station (VOL3), detected an amount of dioxin residues double than the detection threshold (2.55×10^{-6} mg I-TEF/Kg compared to 1.0×10^{-6} mg I-TEF/Kg, where TEF = Toxicity Equivalent Factor). There were no dioxin residues above the LOD in a honey bee samples collected in the Volturno area (table 5).

Conclusion

This study confirms the efficacy of honey bees in detecting environmental contaminants, such as pesticides used in agriculture, and pollutants from other source, such as dioxins. Most importantly is that honey bees, unlike other environmental monitoring methods, capture and assimilate the bioavailable fraction of the pollutants.

The main compounds found were the organophosphorus, primarily in the area Volturno River, in which there were crops of corn, wheat, tobacco and orchards. The anomalous bee mortality and the large number of residues found, detected in honey bees from both areas of research (Volturno River and Sarno River), might have been caused by the improper use of highly toxic and hazard products for bees (i.e. treatments during bloom, non-mowing of the wild flora, presence of wind which caused drifts). We often found residues of such agrochemicals that could refer to a traditional and irregular pest management. The environment appears particularly polluted in the monitoring station of San Marzano sul Sarno (SAR2). Also in the zone of Cancello ed Arnone (VOL3) an intense environmental pressure was present, especially in the most intense agricultural seasons.

The dioxin residues found in the wax samples indicate that honey bee wax can be effectively used as a dioxin accumulator and could therefore provide valid evaluations on the pollution of a well-defined territory. Regarding the amount of the dioxin detected (2.55×10^{-6} mg I-TEF/Kg), since a reference wax value doesn't exist, we could consider that in the underground waters the maximum acceptable value is 4.0×10^{-6} mg I-TEF/Kg (D.M. n. 471, 1999) and in the animal fat is 2.0×10^{-6} mg I-TEF/Kg (Reg. CE 1881, 2006) as well as in the milk and dairy products. Even in this case the bees detected the presence of dangerous molecules confirming by other investigations (Menegozzo *et al*., 2008). This further validates the effectiveness of the use of "honey bees monitoring stations" as an inexpensive alarm and investigation system for researching the human impact on an ecosystem.

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