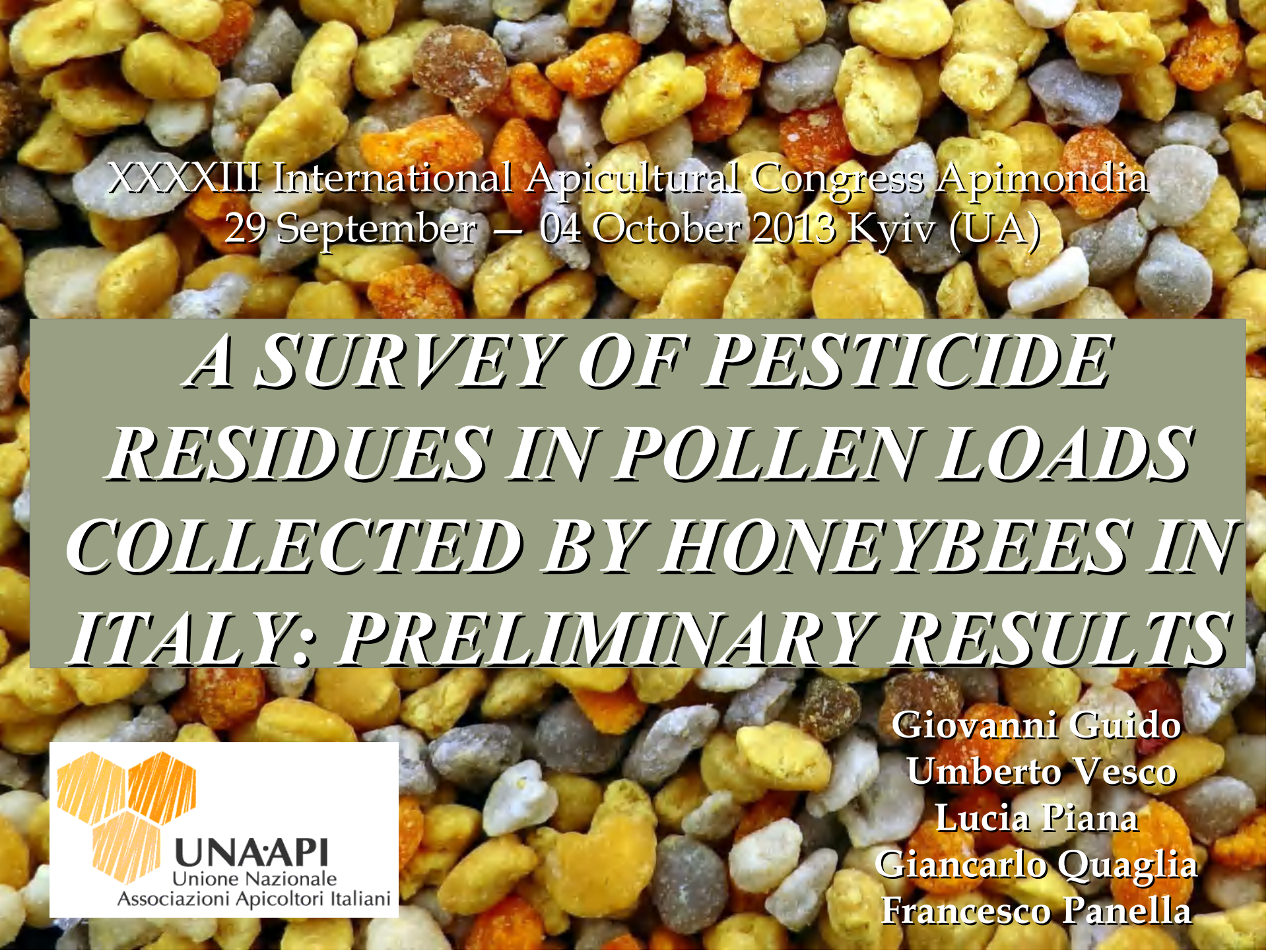

XXXXIII International Apicultural Congress Apimondia
29 September – 04 October 2013 Kyiv (UA)

A SURVEY OF PESTICIDE RESIDUES IN POLLEN LOADS COLLECTED BY HONEYBEES IN ITALY: PRELIMINARY RESULTS

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Introduction

Honeybee populations and other pollinators have declined worldwide in recent years. Various stressors have been hypothesized as potential causes, but there is a growing concern that the widespread use of agricultural pesticides is contributing to the decline of pollinators. Pollen is the main dietary source of proteins, amino acids and lipids and its diversity is essential to bee development and immune system functionality. Pollen is a chemically complex matrix, which can carry into the hive a variety of pesticide residues.

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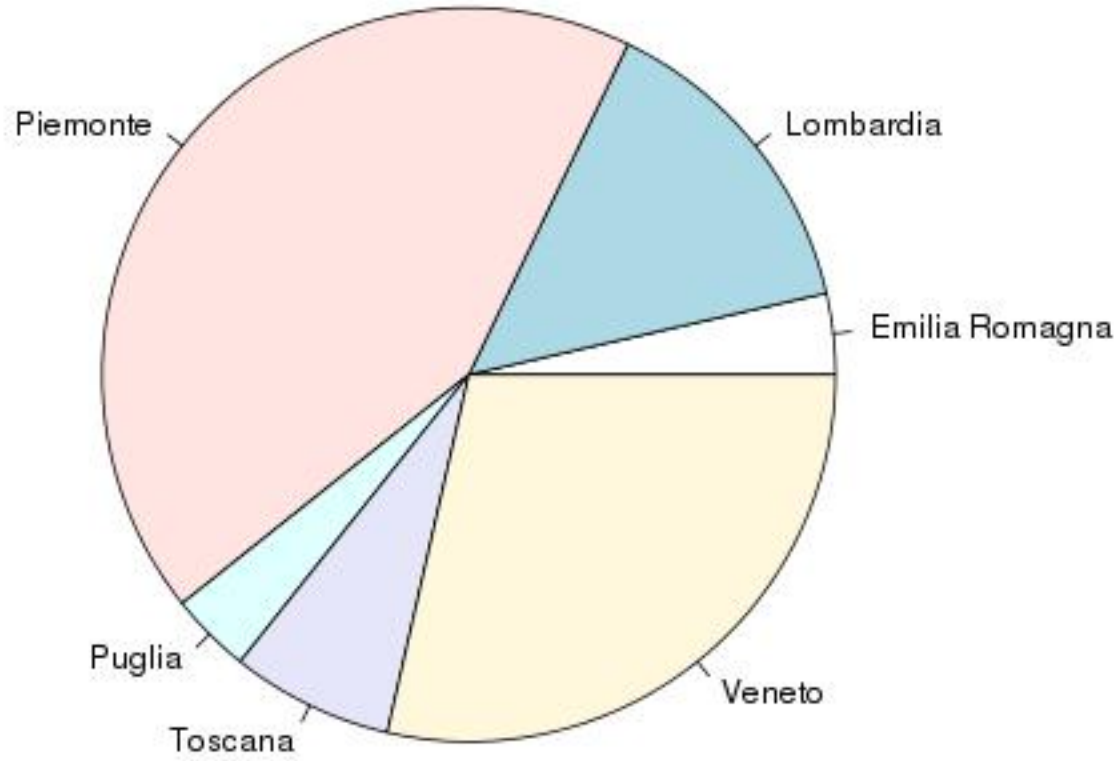
Materials and methods

28 commercial bee yards

Materials and methods

Region	Bee yards
Emilia-Romagna	1
Lombardia	
Piemonte	
Puglia	
Toscana	
Veneto	



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Materials and methods

Pollen collection

Pollen was gathered weekly from 2 colonies with pollen traps

Samples series covered pollen importation season

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Materials and methods

Pollen samples from 2 colonies were merged and immediately frozen

Pollen samples were divided in 3 parts:

- **30g to palynological analysis**
- **100g to multiresidual analysis**
- **100g to control**

Analytical Methods

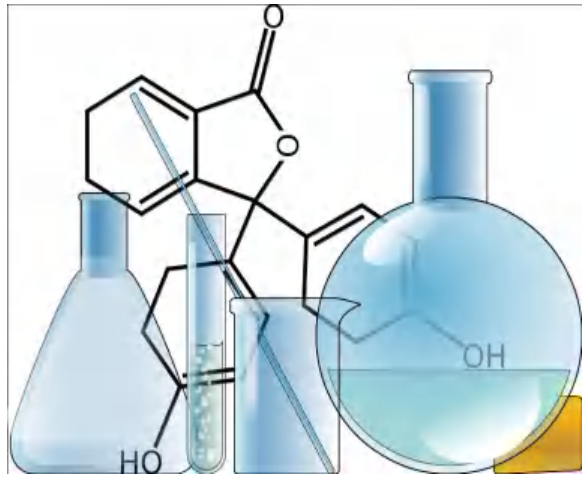
Pollen Sample (10g) was extracted by Acetonitrile, purified by L/L and SPE (Liquid/Liquid, Solid Phase Extraction)

Final concentrated extract were injected into LC-MS/MS (liquid chromatography tandem mass spectrometry) instrument programmed in MRM (Multiple Reaction Monitor) mode (two transition / molecule)

Method validation was carried out according to European Union Directive 2002/657/CE at concentration level of 0.5-1-5 µg/kg depending on sensitivity of molecules

Materials and methods

Multiresidual analysis



We searched residues of 65 pesticides:

- 7 acaricides (4 used by beekeepers)
- 31 insecticides
- 25 fungicides
- 2 herbicides

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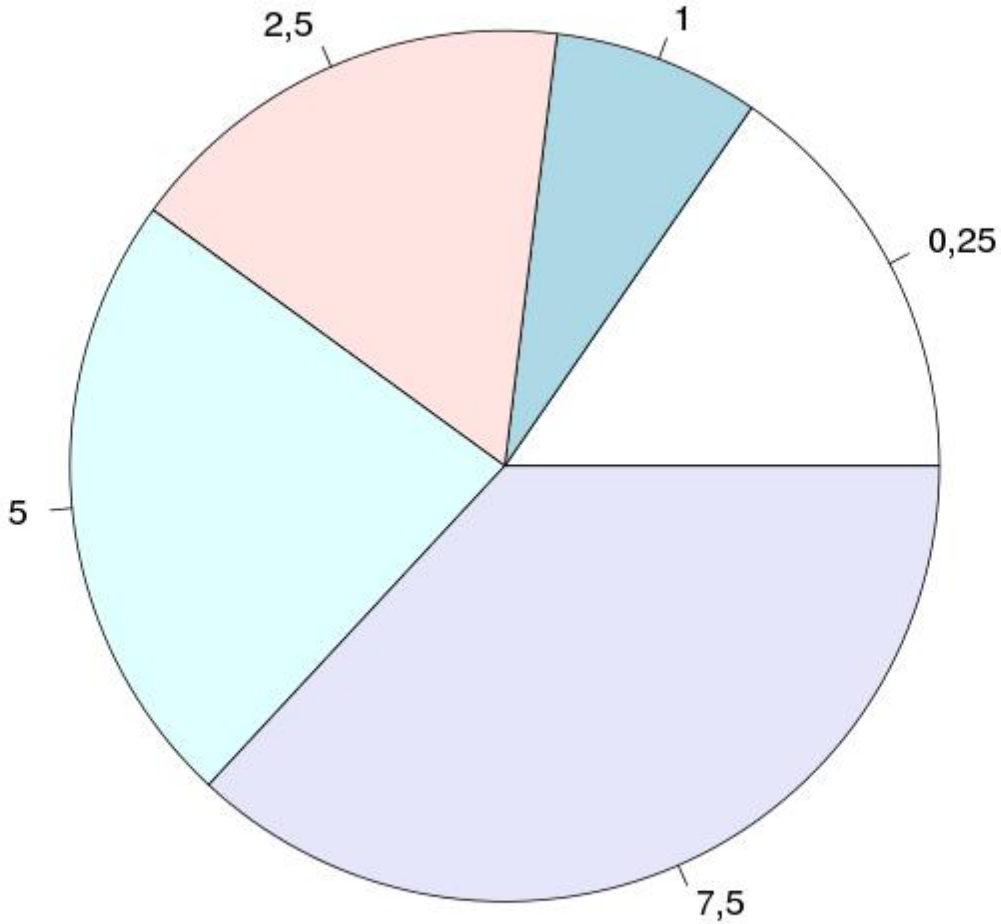
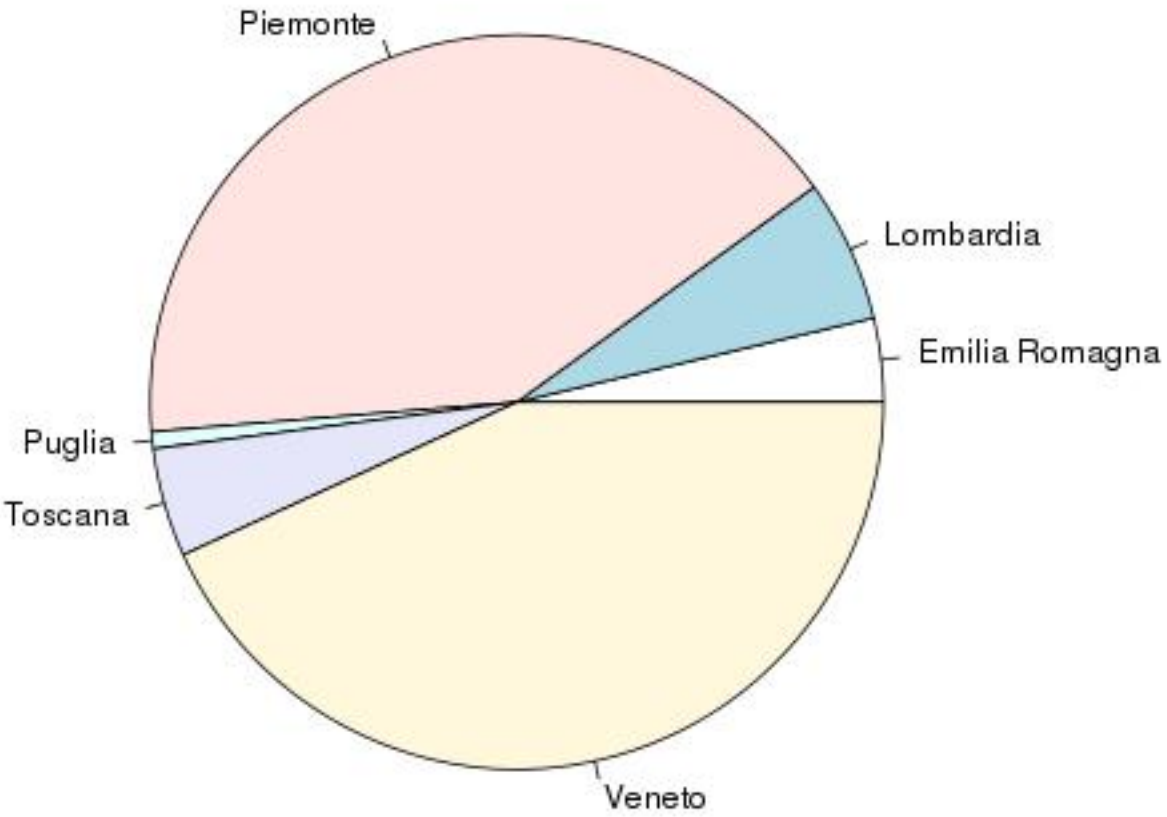
Materials and methods



Results

271 pollen samples

Region	Pollen samples
Emilia Romagna	10
	17
	112
	2
	13
	117



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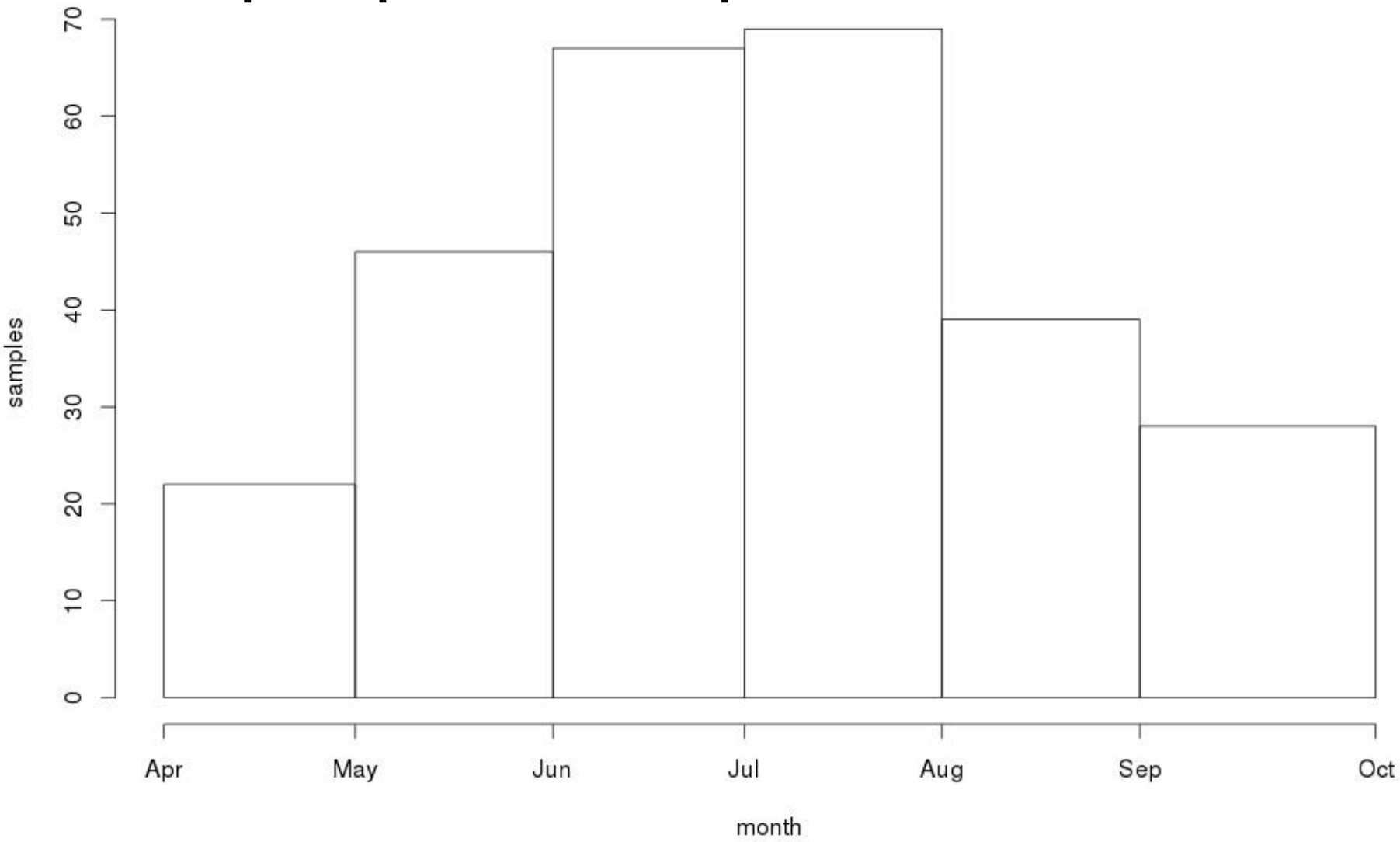
Results

Temporal pattern of samples



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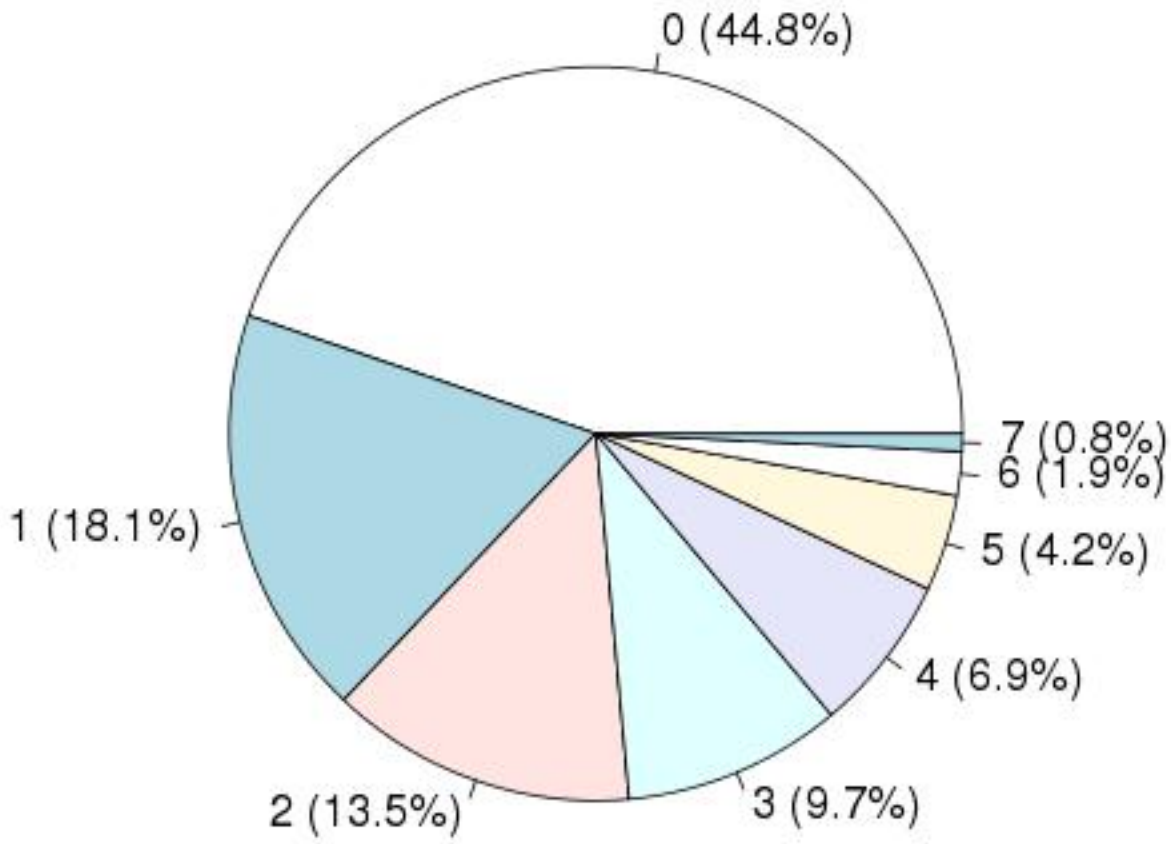
Results

Number of residues contaminating the samples



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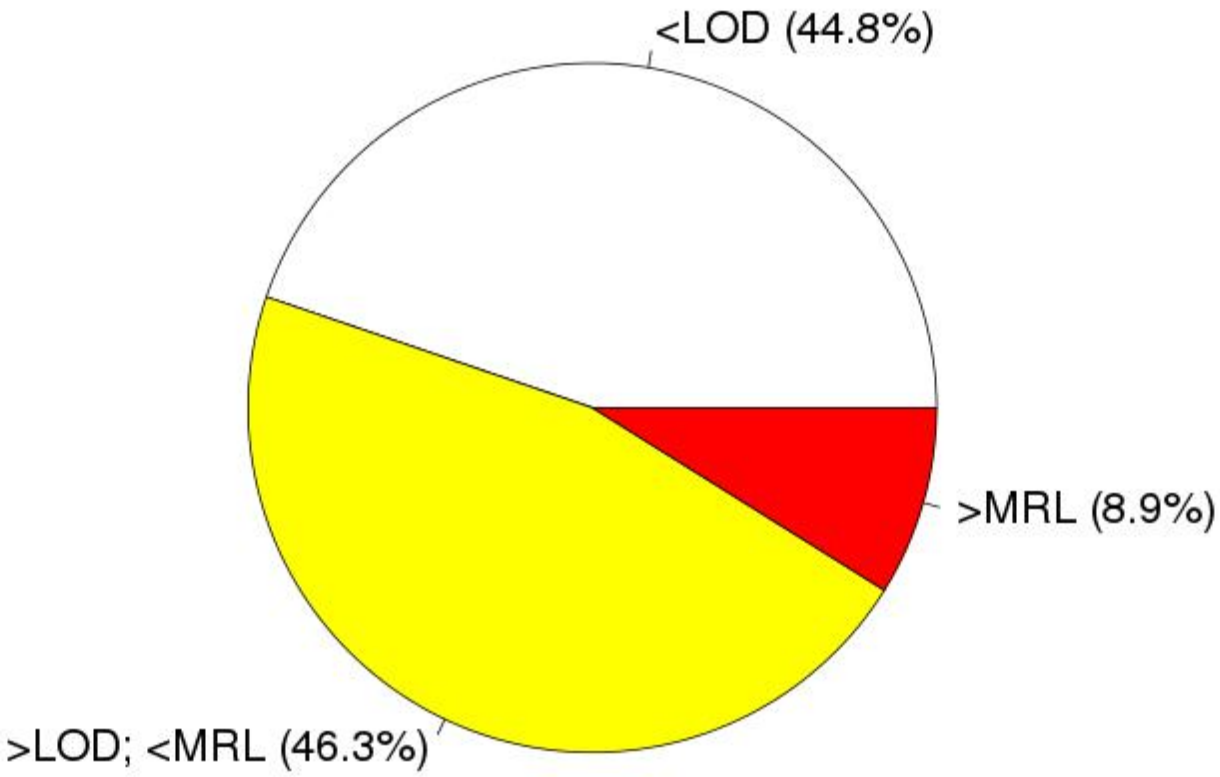
Results



23 samples with residues above MRL for at least 1 pesticide

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Results

13 pesticides found:

- **6 fungicides:**
  - Azoxystrobin
  - Benalaxyl
  - Iprovalicarb
  - Mandipropamid
  - Metalaxyl
  - Spiroxamine

- **6 insecticides**
  - Carbaryl
  - Chlorpyrifc
  - Dimethoate
  - Imidacloprid
  - Phenthoate
  - Thiametoxar

- **1 acaricide:**
  - Chlorfenvinphos

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Results

	>LOD	>MRL	mean	st.dev.	max	min
Mandipropamid	70	6	11,42	34,90	261,2	0,32
Spiroxamine	54	2	3,55	14,64	107,7	0,25
Metalaxyl	47	8	116,03	405,55	2462,6	1,2
Benalaxyl	38	4	4,52	9,86	45,4	0,26
Chlorpyrifos	34	3	4,86	6,70	36,9	1
Chlorfenvinphos	28	4	4,23	8,44	39,37	0,23
Imidacloprid	26	0	2,88	4,89	20,7	0,3
Dimethoate	18	1	10,93	38,08	163,3	0,25
Phenthoate	17	0	0,44	0,95	3,3	0,1
Thiametoxam	16	0	0,84	0,79	2,74	0,19
Azoxystrobin	9	3	11,36	17,19	54	0,2
Iprovalicarb	5	0	1,68	0,58	2,5	1,2
Carbaryl	1	0	0,51	NA	0,51	0,51

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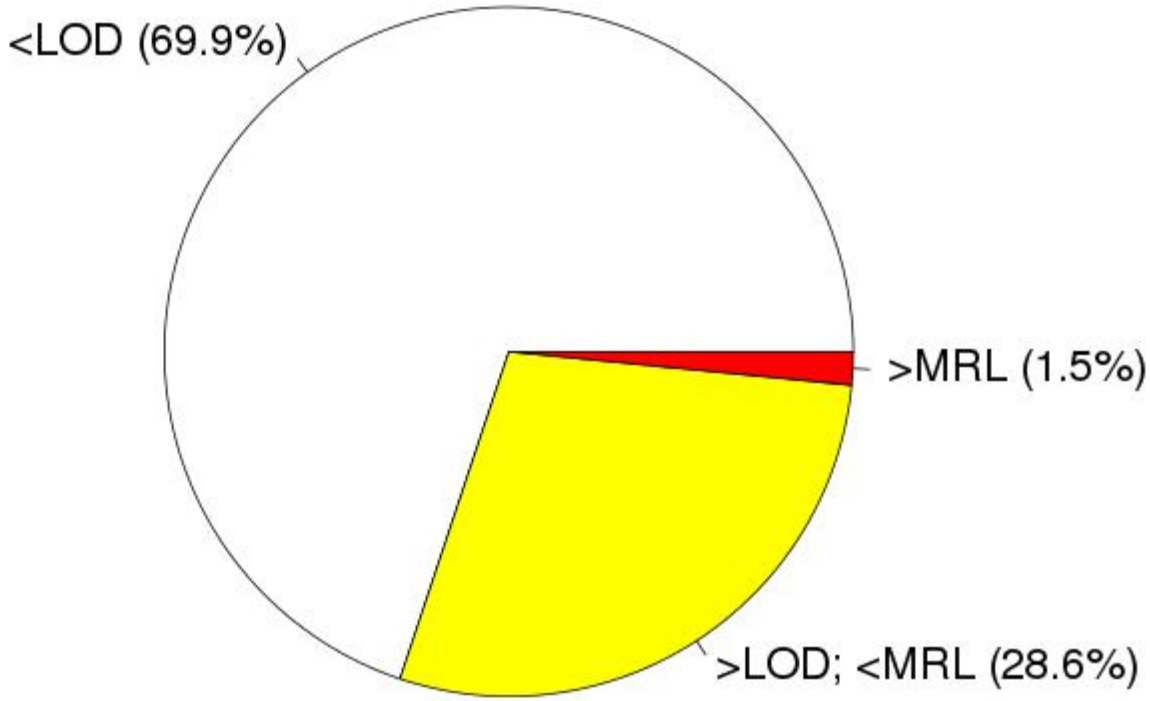
Results

Insecticides



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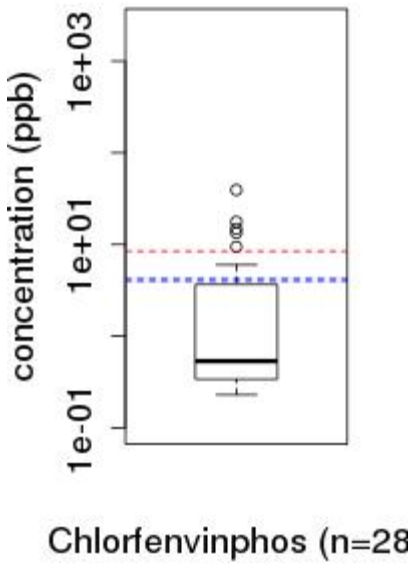
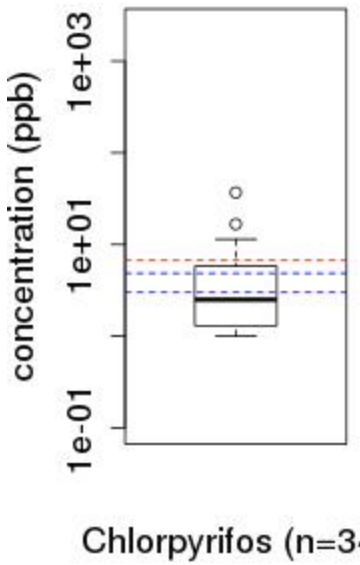
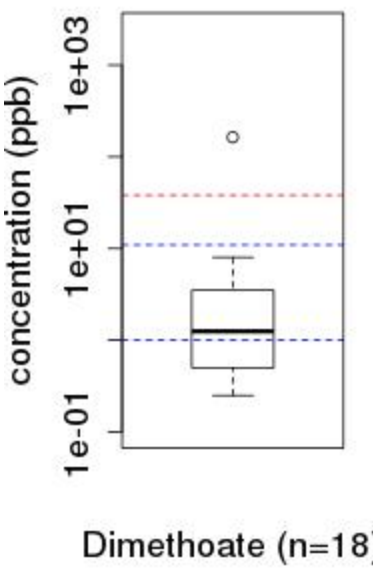
Results



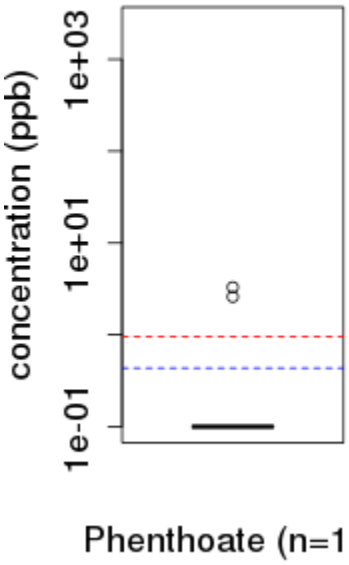
Chlorfenvinphos (n=28



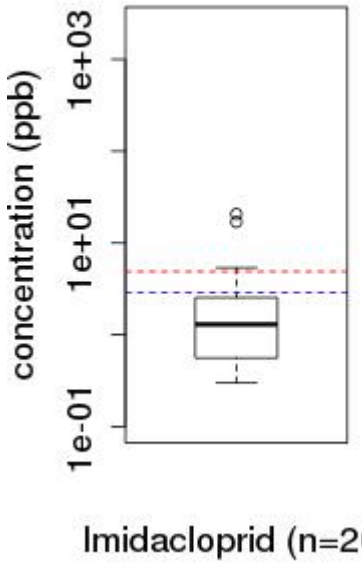
Chlorpyrifos (n=34)



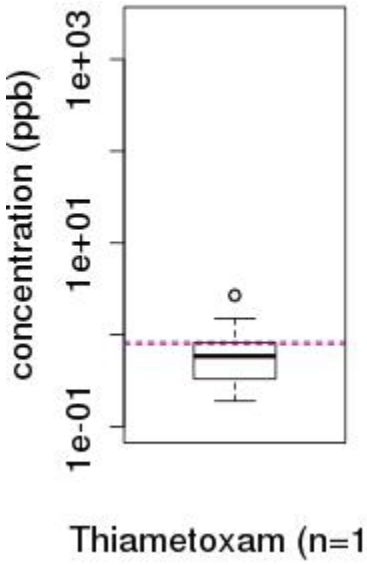
Dimethoate (n=18)



Phenthoate (n=17)



Imidacloprid (n=26)



Thiametoxam (n=16)

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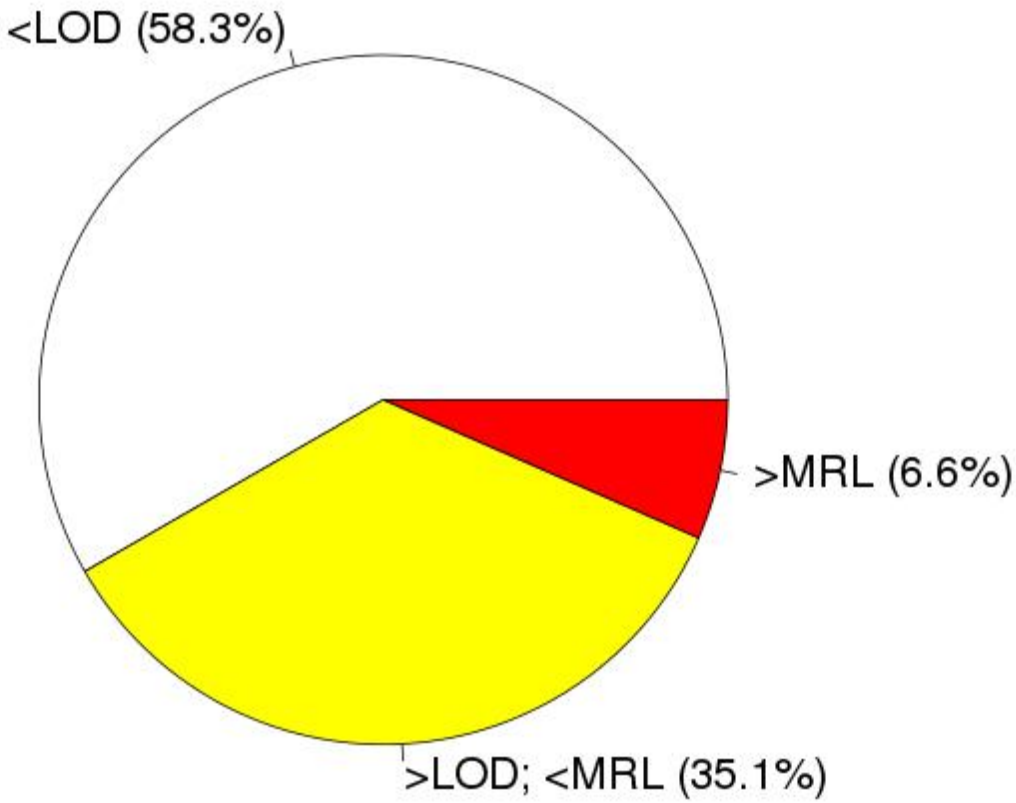
Results

Fungicides



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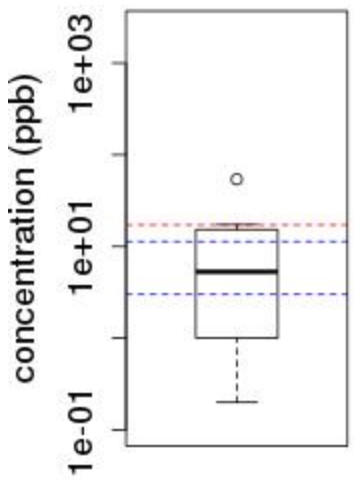
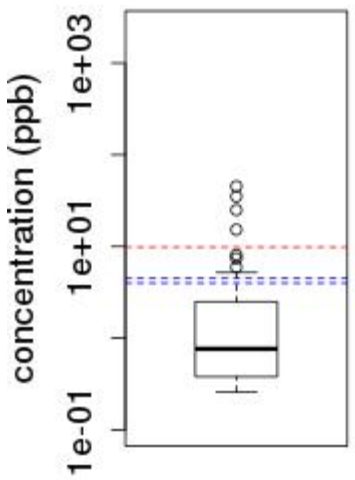
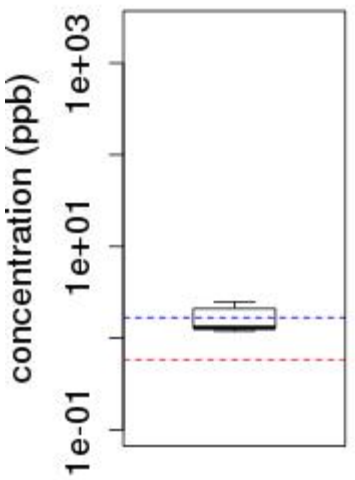


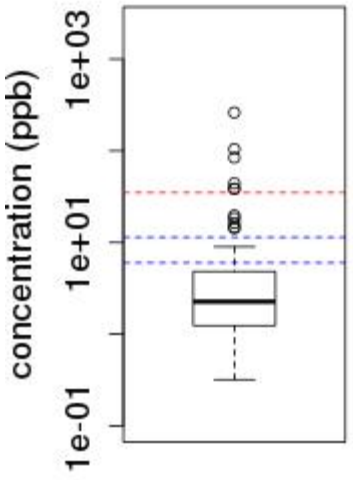
Azoxystrobin (n=9)



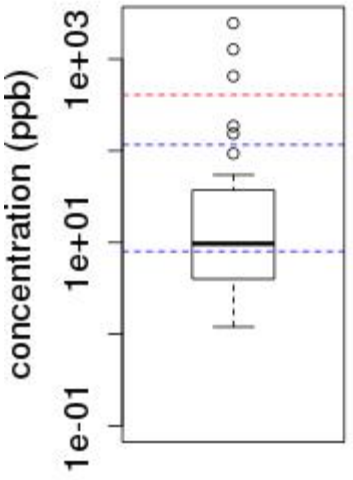
Benalaxyl (n=38)



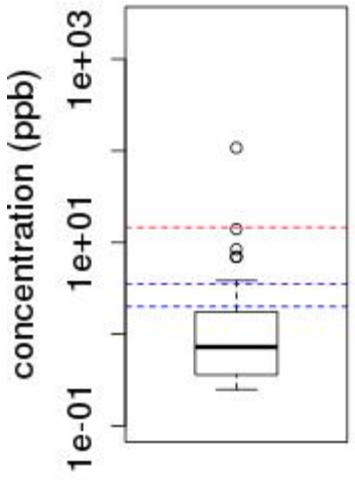
Iprovalicarb (n=5)



Mandipropamid (n=70



Metalaxyl (n=47)



Spiroxamine (n=54)

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Discussion

Widespread contamination raise concerns:

-Effects on bee health (e.g. interaction with immune system / pathogens like *Nosema:* Alaux *et al.* 2010, Pettis *et al.* 2012)

-Damage for beekeepers who commercially produce pollen

Pettis, J. S., vanEngelsdorp, D., Johnson, J. & Dively, G. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenschaften 99, 153–158 (2012).
Pettis, J. S. et al. Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae. PLoS ONE 8, e70182 (2013).
Alaux, C. et al. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera). Environmental microbiology 12, 774–782 (2010).

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Discussion

Widespread fungicide contamination of pollen: possible sub-lethal effects

-inibition of bee bread methabolism (Yoder *et al.* 2013, Heydinger *et al.* 2011*)*

-interaction with varroacides and insecticides e.g. CitP450 competition (Johnson *et al.* 2013), thermoregulation (Vandame & Belzunces 1998)

-fungicide contamination has been related to increased mortality (vanEngelsdorp *et al.* 2009)

Yoder, J. A. et al. Fungicide Contamination Reduces Beneficial Fungi in Bee Bread Based on an Area-Wide Field Study in Honey Bee, Apis mellifera, Colonies. Journal of Toxicology and Environmental Health, Part A 76, 587–600 (2013).

Vandame, R. & Belzunces, L. P. Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation. Neuroscience Letters 251, 57–60 (1998).

Derrick Heydinger et al. in Honey Bee Colony Health 193–214 (CRC Press, 2011). at <http://dx.doi.org/10.1201/b11318-18>

vanEngelsdorp, D. et al. 'Entombed Pollen': A new condition in honey bee colonies associated with increased risk of colony mortality. Journal of Invertebrate Pathology 101, 147–149 (2009).

Johnson, R. M., Dahlgren, L., Siegfried, B. D. & Ellis, M. D. Acaricide, Fungicide and Drug Interactions in Honey Bees (Apis mellifera). PLoS ONE 8, e54092 (2013).

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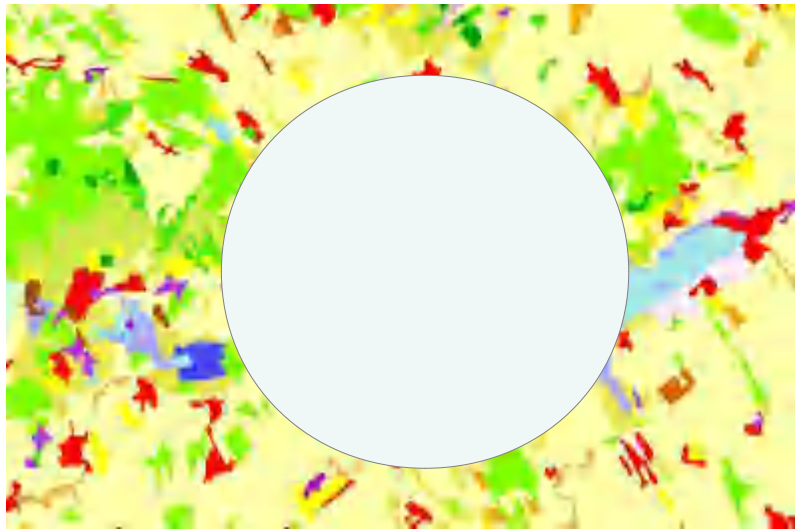
Discussion

Looking forward...

Still collecting data from Southern Italy

To look at possible correlations between land use, palynology results and pollen contamination (GIS)



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