Science of the Total Environment 615 (2018) 208–218

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides

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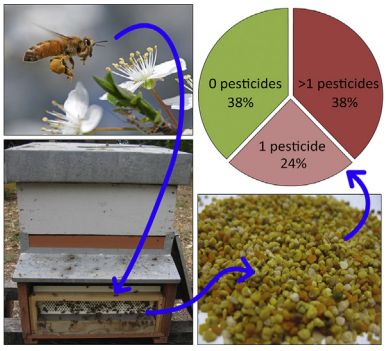
HIGHLIGHTS

- The majority (62%) of pollen samples contained at least one pesticide (2012–2014).
- Multiresidual samples (38%) were more frequent than single contaminations (24%).
- Chlorpyrifos was the most frequently detected pesticide (30%).
- Imidacloprid-contaminated samples had the highest HQ, with 12% of samples >1000.
- Health safety levels (ARfD, ADI, MRL) were exceeded in 39% of the residues.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 August 2017
Received in revised form 20 September 2017
Accepted 21 September 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Bee health
Monitoring
Multiple residues
Chemical mixture
Chlorpyrifos
Imidacloprid

ABSTRACT

Honey bee (*Apis mellifera* L.) health is compromised by complex interactions between multiple stressors, among which pesticides play a major role. To better understand the extent of honey bee colonies' exposure to pesticides in time and space, we conducted a survey by collecting corbicular pollen from returning honey bee foragers in 53 Italian apiaries during the active beekeeping season of 3 subsequent years (2012–2014).
Of 554 pollen samples analysed for pesticide residues, 62% contained at least one pesticide. The overall rate of multiresidual samples (38%) was higher than the rate of single pesticide samples (24%), reaching a maximum of 7 pesticides per sample (1%). Over 3 years, 18 different pesticides were detected (10 fungicides and 8 insecticides) out of 66 analysed. Pesticide concentrations reached the level of concern for bee health (Hazard Quotient (HQ) higher than 1000) at least once in 13% of the apiaries and exceeded the thresholds of safety for human dietary intake (Acute Reference Dose (ARfD), the Acceptable Daily Intake (ADI), and the Maximum Residue Limit (MRL)) in 39% of the analysis. The pesticide which was most frequently detected was the insecticide chlorpyrifos (30% of the samples overall, exceeding ARfD, ADI, or MRL in 99% of the positive ones), followed by the fungicides mandipropamid (19%), metalaxyl (16%), spiroxamine (15%), and the neonicotinoid insecticide imidacloprid (12%). Imidacloprid had also the highest HQ level (5054, with 12% of its positive samples with HQ higher than 1000).
This 3 year survey provides further insights on the contamination caused by agricultural pesticide use on honey bee colonies. Bee-collected pollen is shown to be a valuable tool for environmental monitoring, and for the detection of illegal uses of pesticides.

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https://doi.org/10.1016/j.scitotenv.2017.09.226
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1. Introduction

In the last century agriculture has expanded and intensified (Ramankutty and Foley, 1999), providing higher crop yields for a growing world population. The increased agricultural practices however have had a high environmental cost: habitat loss and widespread use of pesticides have posed significant negative consequences for wild flora and fauna (Matson et al., 1997; McLaughlin and Mineau, 1995; Van Dijk et al., 2013). Thus, it is not surprising that there is an ongoing global decline of pollinators (Biesmeijer et al., 2006; Potts et al., 2010), which is alarming due to the important role pollinators play in ecological systems and crop productivity (Aizen et al., 2009; Fontaine et al., 2005; Garibaldi et al., 2011; Klein et al., 2007). Honey bees, important crop pollinators, can be considered as indicators of the health status of pollinating insects. In fact, because beekeepers rear and monitor bee colonies worldwide, they are immediately aware of changes in colony health, productivity, and behaviour. Indeed, it was beekeepers who alerted the media and scientific community about an increase in the normal rate of colony mortality around 2006 (Cox-Foster et al., 2007). The phenomenon was named Colony Collapse Disorder (CCD) or more generically "colony losses", and it engendered research initiatives across the world (Carreck and Neumann, 2010).

Various stressors have been investigated and found to be possible cause of the phenomenon: parasites and pathogens (Cornman et al., 2012; Cox-Foster et al., 2007; Dainat et al., 2012; Higes et al., 2010; Le Conte et al., 2010; Ravoet et al., 2013), pesticides (Belzunces et al., 2012; Desneux et al., 2007; Sandrock et al., 2014), climate change (Memmott et al., 2007) and nutrition (Archer et al., 2014). Much of the evidence collected in recent years suggests that a combination of these factors, acting in synchrony and with complex interactions, is responsible for the increased honeybee colony mortality. Pesticides are considered to be a key factor, as a multitude of studies have demonstrated their detrimental effects at both individual and colony level (Goulson, 2013; Sánchez-Bayo et al., 2016; Sgolastra et al., 2017b; Tosi et al., 2017; van der Sluijs et al., 2013). Many of these studies were conducted in vitro and/or in semi-field conditions and their results were questioned because of the lack of certainty about the actual pesticide exposure of bees in the field (Blacquière et al., 2012). However, recent studies have addressed the problem at the field level and have confirmed the detrimental effects of pesticide exposure for bees (Rundlöf et al., 2015; Woodcock et al., 2017). Furthermore, they have shown that a realistic scenario comprehends a continuous exposure to multiple pesticides (Botías et al., 2017; David et al., 2016; Long and Krupke, 2016). Because of the prolonged exposure to the toxins, this kind of contamination may be more harmful to honey bees than pulse exposures which are normally tested in laboratory conditions (Laycock and Cresswell, 2013).

Several of the most commonly used pesticides are systemic, protecting (and contaminating) all plant organs, including flowers—and thus nectar and pollen. Pollen is the main protein and lipid source for bee colonies and a fundamental part of the nurse bees' and larval diet (Crailsheim et al., 1992), thus its contamination results in exposure of the new generation of bees, as well as the foraging and receiver bees. Some studies already evidenced widespread contamination of pollen from agricultural landscapes, and highlighted common combinations of pesticides encountered in field environments (Bernal et al., 2010; Chauzat et al., 2006; Lambert et al., 2013; Long and Krupke, 2016; Mullin et al., 2010; Smodis Skerl et al., 2010). Advocates of chemical plant protection claim that if the products are used according to good agricultural practices the effect on the environment should be negligible (Cutler et al., 2014). However, exposure to low levels of pesticides can elicit sublethal effects on bees, not killing them outright but affecting their behaviour and immune system (Desneux et al., 2007). The detection of residues at very low levels has become possible, in recent years, as new analytical techniques have been developed (Stachniuk and Fornal, 2016).

Foraging honey bees fly to an average distance of about 1.5 km from the colony (Steffan-Dewenter and Kuhn, 2003; Visscher and Seeley, 1982), meaning that an area of approximately 7 km^2 around the hive is visited by foraging bees. The average size of a European farm is 0.16 km^2 (Eurostat, 2012), thus a foraging surface of 7 km^2 is normally covered by several crops, exposing a colony placed in a rural area to multiple pesticides used for different crops. Furthermore, a multitude of pesticides are available, for example Italian farmers have access to approximately 130 different active ingredients (aa.ii.), alone or in combination, in about 1280 commercialized products for plant protection (Ministero del lavoro della salute e delle politiche sociali, 2014).

The aim of this study was to investigate the extent of honey bee exposure to agricultural pesticide residues in managed honey producing colonies. This was achieved by analysing corbicular pollen from returning forager bees (it has been shown that pollen loads are the best matrix for assessing ongoing pesticide contamination in the environment (Chauzat et al., 2011)) and using residue levels to estimate the risk hazard for honey bees. Furthermore, as pollen is also used for human consumption as a "health food supplement" (Campos et al., 2003; Carpes et al., 2009; Graikou et al., 2011; LeBlanc et al., 2009), the obtained results were compared with regulatory agency levels of concern for acute or chronic exposure in humans.

2. Material and methods

2.1. Survey period and sites

We used 53 commercial apiary sites located in Italy (22 apiaries in 2012, 24 in 2013 and 15 in 2014; 8 apiaries were used multiple years) (Fig. 1). A total number of 554 pollen samples were collected between March and September of 3 consecutive years, from 2012 to 2014. Overall, the apiaries were located in proximity of agricultural areas and were randomly selected across Italy based on apiary size, beekeeper's experience and beekeeper's ability to adhere to the working protocol of the survey. Beekeepers experience was estimated based on years of experience, membership in a beekeeping association, and training level (frequency of beekeeping meetings, conferences, workshops, and seminars attended) (EFSA, 2016). About 65% of the beekeepers managed their apiaries according to the organic production protocols (European Council, 2007). Within each apiary, 5 queen-right and healthy (i.e. no disease symptoms) honey bee colonies (*Apis mellifera* L.) were used for pollen collection.

2.2. Pollen collection

Colony management and pollen sampling and shipment were carried out by the beekeepers and apiary technicians. They were provided with a working protocol defining all monitoring details, and were personally instructed by expert beekeepers and ecotoxicologists in ad-hoc meetings to improve the harmonization of the procedure across apiaries and beekeepers.

Commercially available pollen traps were used to dislodge the pollen pellets from the corbiculae of returning foraging bees. The pollen traps were kept in place until 100 g of pollen pellets were collected (typically 2–7 days). The sampling period and success varied in relation to weather conditions and pollen import by the colonies. Samples were collected during the active beekeeping season, in the most critical periods for pesticide contamination (e.g. concomitantly with agricultural pesticide treatments), based on expert experience (i.e. consultation of farmers, beekeepers, and agronomists) on the agricultural practices in their area. After collection, the pollen pellets were homogenized using a glass jar, and 100 g were subsampled and frozen at −20 °C. A cool-box was used for shipment of the samples from the

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