Science of the Total Environment 999 (2025) 180285

Contents lists available at ScienceDirect

Science of the Total Environment

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

Honey bee hives as biomonitors of pesticide environmental pollution. The INSIGNIA-EU monitoring action

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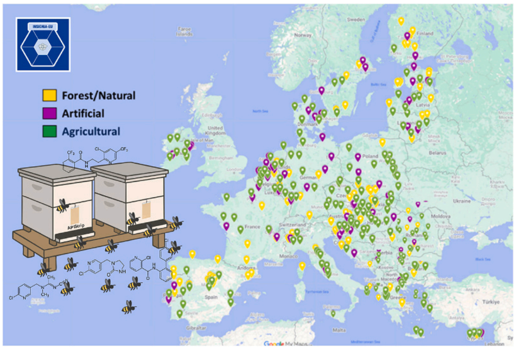
HIGHLIGHTS

- The data presented constitute the first pan-European study on pesticide pollution in terrestrial environments.
- An EU-wide citizen science network of beekeepers collaborated in the extensive sampling campaign.
- APIStrips in beehives have demonstrated their efficacy and robustness for large-scale monitoring campaigns.

GRAPHICAL ABSTRACT



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https://doi.org/10.1016/j.scitotenv.2025.180285
Received 13 May 2025; Received in revised form 4 July 2025; Accepted 14 August 2025

ARTICLE INFO

Keywords:
Pesticide pollution
Terrestrial environments
APIStrip
EU monitoring
Analysis

ABSTRACT

Honey bee hives provide invaluable advantages as effective tools for monitoring pesticides, providing protected environments with consistent temperature, humidity, and airflow. They continuously accumulate pesticides from the surrounding area due to both airflow and honey bee foraging activity, which efficiently transport pesticides to the colony over space and time. This study presents extensive European monitoring data collected using a noninvasive in-hive passive sampler, the APIStrip, which employs TENAX® and is effective at adsorbing pesticides.

As part of the INSIGNIA-EU monitoring action (www.insignia-bee.eu), APIStrips were deployed in georeferenced apiaries across all 27 EU countries. Apiary selection was based on key factors, including agricultural, artificial, and forest/natural land use categories. Sampling was conducted simultaneously across all apiaries over nine consecutive two-week periods from May to August 2023. Of the 429 pesticide compounds targeted in the analysis, 188 were detected in the 5524 APIStrips analyzed.

As expected, agricultural areas presented the highest pesticide levels, with notable variations in the number of compounds detected and amounts found between the apiaries. Azoxystrobin, boscalid, tebuconazole, acetamiprid, and fluopyram were found in more than 50 % of sampling sites evaluated. Overall, and across all sampling rounds, 96.4 % of APIstrips contained at least one pesticide. This study has produced the first EU-wide distribution map of terrestrial pesticide contamination and demonstrates widespread pesticide contamination of EU environments.

1. Introduction

Pesticide use continues to be a significant concern for biodiversity conservation in both agricultural and non-agricultural areas. While pesticide water monitoring has been established at the European scale in response to the EU Water Framework Directive (EUROPEAN, 2025), measurements of pesticides in terrestrial matrices are limited to a few studies that have considered soil (Vieira et al., 2023; Orgiazzi et al., 2022; Silva et al., 2006), vegetation (Cech et al., 2022; Linhart et al., 2021), earthworms (Pelosi et al., 2021), and insects (Honert et al., 2025). Therefore, the presence of pesticides in terrestrial environments and their exposure to non-target organisms remain largely unexplored. This is primarily due to the challenges associated with monitoring large-scale environments which limit the availability of consistent data on the presence of these compounds. Additionally, the extremely low concentrations of individual pesticides, often in the picogram per cubic meter (pg/m^3) range or even lower, pose a significant detection challenge. Despite advances in modern analytical methods, such low levels remain undetectable in most cases.

Active air sampling (Brüggemann et al., 2024; Szczurek et al., 2021; Wania et al., 2020; Udomkun et al., 2023; Kruse-Plaß et al., 2021) for these contaminants typically requires sampling volumes of several hundred or even several thousand m^3 to allow for reliable detection and quantification. Active air samplers are expensive and complex, requiring power sources, regular maintenance, and calibration. Thus, passive samplers (PASs) appear to be an alternative excellent alternative solution, as they have very low prices, simple to operate and not require power sources.

However, some drawbacks are that high sampling volumes are not attainable with classical PASs and they often exhibit high uncertainty when correlating detections with actual sampled amounts. This uncertainty is further amplified in pesticide monitoring due to varying pesticide use regimes, which significantly influence the variability of pesticide presence across landscapes.

To effectively link real-world pesticide presence, an empirical, landscape-scale approach is essential. Understanding when, where, and how many pesticides are present in the environment is a crucial first step toward quantifying exposure levels and assessing the potential for co-exposure to multiple pesticides in non-target organisms.

The honeybee (*Apis mellifera* L.) colonies have been used to monitor environmental contaminants such as pesticides, heavy/toxic metals etc. for biomonitoring purposes (Raeymaekers, 2006; Conti et al., 2022; Stöcker, 1980; Balestra et al., 1992; Marcoccia et al., 2024; Cunninghama et al., 2022; Mair et al., 2023; Girotti et al., 2020; Papa et al., 2024). Each honey bee can act as an environment microsampler, with a honey bee colony as a sampler unit. However, invasive sampling methods such as sampling live bees for analysis present a significant drawback, as they adversely affect bee populations and deter beekeepers who might participate in monitoring programs. This issue makes it impractical to scale up these monitoring practices across extensive areas and over time. Non-invasive passive samplers thus provide an alternative.

The ambition of the INSIGNIA-EU Action was to establish a citizen scientist pan-EU network using beekeepers to bio monitor environmental pollution using honey bee colonies across the EU. This monitoring initiative engaged 315 citizen scientist beekeepers across all 27 EU countries, following a standardized protocol based on the effective and non-invasive APIStrip passive sampler (Murcia-Morales et al., 2020; Murcia-Morales et al., 2023; Murcia-Morales et al., 2021a; Murcia-Morales et al., 2021b). The study was conducted over nine consecutive two-week periods from April 20 to August 24, 2023, ensuring simultaneous data collection and thus direct comparability of results.

2. Materials and methods

2.1. Passive samplers: "APIStrips"

The preparation of the APIStrip samplers was performed according to the protocol described in a previous study (Murcia-Morales et al., 2020). Briefly, a polystyrene rectangle (5 × 10 × 0.2 cm) was coated with a TENAX® solution in dichloromethane (6 ml, 125 mg/ml), resulting in 0.75 g of sorbent per APIStrip. The upper section was left uncovered to facilitate handling, and the samplers were not handled with bare hands in order to avoid cross-contamination. The APIStrips were stored individually, wrapped in aluminum foil inside sealed bags. After shipment to the apiaries, they were subsequently inserted into the bee hives, where they remained for 14 ± 2 days. In each apiary, we installed two APIStrips simultaneously in two beehives. This procedure was repeated across nine consecutive sampling events at each apiary. The APIStrip collections from each country were centralized by a national coordinator, stored at 4 °C in a refrigerator, and shipped for analysis every three sampling rounds according to the INSIGNIA-EU standardized citizen-science protocol. Field blanks were also shipped to each beekeeper to assess potential contamination: these were treated, stored, and analyzed in the same way as the real samples, but they were not placed into bee hives. Additionally, blank APIStrips remained in the laboratory and were analyzed with each set of samples to assess in-house contamination. It should be noted that the PAS sampling system based on TENAX® has limited efficiency in trapping highly polar pesticides, which may lead to an underestimation of their contribution.

2.2. Sample collection

The INSIGNIA-EU project aimed to collect environmental monitoring data across a wide area of the European Union, covering diverse types of landscapes. To classify these landscapes, the project used the 2018 CORINE Land Cover data (https://land.copernicus.eu/en/products/corine-land-cover/clc2018), categorizing areas into three main land use types: agricultural, artificial (urban, industrial, or infrastructure), and natural/forest. For each apiary, the dominant land use within a 3 km radius was identified. In most selected locations, other land use types contributed only minimally.

To ensure a balanced sample, national coordinators recruited beekeeper citizen scientists from across the different land use categories while also aiming for broad geographical distribution. For practical reasons, most participating beekeepers were located in agricultural areas, with fewer in artificial or natural/forest regions. Despite these limitations, the overall distribution of apiaries as 54 % Agricultural, 21 % Artificial and 25 % Forest was considered sufficiently representative for the project's objectives. It is important to note that CORINE does not set a fixed percentage threshold for 'dominant' in practice, however, the dominant class typically occupies well over 50 % of the area. (See Fig. 1.)

2.3. Chemical analysis

After the sampling period, the APIStrips were shipped back to the laboratory and chemical contaminants were desorbed following previously developed and optimized procedures (Stöcker, 1980; Balestra et al., 1992; Marcoccia et al., 2024). The extraction involved cutting each sampler into small pieces that were placed in 50-ml PTFE centrifuge tubes; then, surrogate standards (dichlorvos-D6, malathion-D10, parathion-D10, carbendazim-D3) were added, followed by 10 ml of acetonitrile. The samples were automatically shaken for 3.5 min and centrifuged for five minutes at 4000 rpm. Two aliquots of each sample (0.5 ml) were evaporated to dryness and reconstituted with 50 µl of the injection solvent (acetonitrile for liquid chromatography -LC-, ethyl acetate for gas chromatography -GC-). Lindane-D6 and dimethoate-D6 were added as injection internal standards for GC and LC, respectively, as well as 200 µl of ultrapure water to the LC vials. The analyses by GC–MS/MS were performed in an Agilent Intuvo 9000 GC system coupled to an Agilent 7010B GC MS/MS triple quadrupole, while an Agilent UPLC 1290 Series coupled to an Agilent 6490 Triple Quad LC/MS was employed for the LC-MS/MS analyses. The analytical methods have been previously described (Murcia-Morales et al., 2020). A detailed list of the contaminants included in the analytical scope, as well as the transitions and their limits of quantification, can be found in the Supplementary Material (Annex I).

The identification of the 429 pesticide residues included in the present study was performed according to the guidelines established by the SANTE Document No. 11312/2021 (Unknown, 2023): Two fully overlapping transitions with a signal-to-noise ratio (S/N) higher than 3, whose ion ratio in the sample is within ±30 % from the average of the calibration standards, and a retention time in the sample within ±0.1 min from that of the calibration standards. Recovery studies at 2 or 5 ng/APIStrip for all compounds were performed and described in a previous work, and the recovery of more than 98 % of compounds fell within the range 70–120 %. The minimum matrix matched standard calibration point that allowed the identification of each compound according to the criteria described above was set as the limit of quantification (LOQ). The LOQ for most compounds was determined to be 0.5 ng/APIStrip. Positive detections below the LOQ that were fully identified were assigned to a default limit of detection (LOD) value of 0.25 ng/APIStrip. The detailed target list, the LOQs, and the transitions used for the identification of all target compounds are provided in the Supplementary Material (Annex I).

3. Results and discussion

3.1. Preliminary considerations

The passive samplers, the APIStrips, installed in the hives can capture pesticides originating both from the surrounding environment and from within the hive itself (Cunninghama et al., 2022). Internal sources may include veterinary treatments applied by beekeepers into the hive to control parasitic varroa mites, or may be present in the hive via contaminated wax as well as from disinfectants or biocides used on hive materials, such as wood preservatives or moth repellents. Mixing these two sources, environmental and internal to the hive, can lead to undesirable artifacts and potentially inaccurate interpretation of the detected compounds.

Although the INSIGNIA project included 450 compounds, these chemicals of internal hive origin, along with highly polar and high water

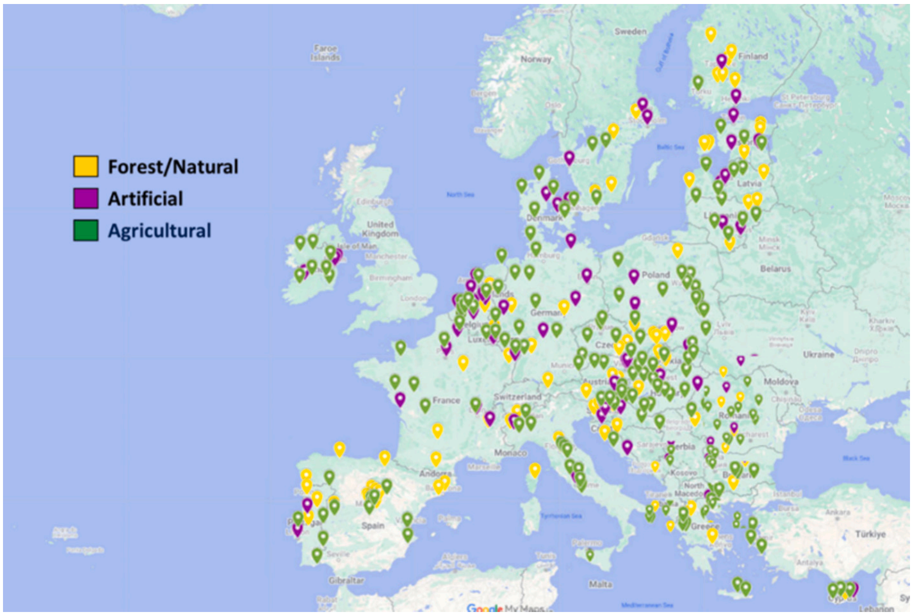

Fig. 1. Selected sample distribution across the 27 EU member states.

soluble pesticides; glyphosate, AMPA, ethephon and fosetyl aluminum (which exhibit low adsorption on TENAX®) are excluded from the subsequent result analysis to ensure a more accurate evaluation of external environmental exposure, resulting in an effective target list of 429 compounds.

Additionally, DEET was included in the target list, even though it is classified as a biocide/repellent rather than a pesticide. Its inclusion was considered valuable as a reference for the presence of a compound with significant anthropogenic use. It was only included in the concentration graphs to illustrate the impact of a widely used compound across all of the land use categories.

3.2. *Pesticide detection*

This total of 429 target compounds were monitored over two-week exposure periods using APIStrips. Of these, 188 compounds were actually detected in the 5524 APIStrips analyzed; representing 97.4 % of the APIStrips distributed to beekeepers. The total number of detections was 17,213 above the Limit of Detection, considering full identification (LOD) of 0.25 ng per APIStrip. There was no sample site where there was no pesticide occurrence over the complete sampling period (Annex II). These findings reveal that pesticide residues are ubiquitously present across the EU environment. Recent monitoring efforts using bee matrices such as bee bread, propolis and beeswax have shown comparable detection results (Vegh et al., 2023). However, substantial differences in study scope and sampling methodologies limit the feasibility of a detailed comparison.

Four pesticides: acetamiprid (insecticide); azoxystrobin (fungicide); boscalid (fungicide); and tebuconazole (fungicide) were detected in all 27 EU countries in more than 50 % of the sampling sites.

The 31 most frequently detected pesticides (Annex III) across all EU countries were selected based on their occurrence in more than 40 sampling sites (approximately 10 % of the total) in all sampling rounds, and with more than 100 individual detections. Listed in order, they are as follows: azoxystrobin; acetamiprid; boscalid; fluopyram; tebuconazole; permethrin; cypermethrin; acrinathrin; pyraclostrobin; imidacloprid; trifloxystrobin; chlorfenvinphos; mandipropamid; fluxapyroxad; fludioxinil; difenoconazole; pyriproxyfen; chlorpyrifos; thiabendazole; metolachlor; fipronil carbendazim; cyprodinil; ametoctradin; thiacloprid; dimethenamid; metconazole; deltamethrin; propamocarb; chlorantraniliprole and pendimethalin. Of these, 16 are fungicides, 12 are insecticides, and 3 are herbicides. Together, these 31 compounds account for more than 71 % of the total number of detections. It is important to note that two APIStrips were placed in each sampling site, using two hives (see Materials and Methods), and that detections from both strips were summed.

Notably, the number of detections recorded by the two APIStrips was very similar, typically differing by less than 7 %, with an average overall difference of only 2.8 % in the number of detected compounds when considering the two strips across the full dataset. This result highlights the reliability of the PAS sampling procedure.

3.3. *Spatial and temporal variations*

Spatial and temporal differences were observed, with regions exhibiting varying patterns both within and across countries. The median number of detections of pesticides per apiary was 87 (Fig. 2). A large amount, as expected, of spatial variation was observed, ranging from just 4 detections over all sampling rounds in the Austrian apiary “AT07”, located in an area with artificial land use, to 250 detections in the Polish sampling site “PL08”, situated in an agricultural landscape (see Annex II). Even within the same country, such as Poland, the difference is striking; from 250 detections in “PL08”, to only 7 in the apiary “PL07”, where the land use is artificial. Therefore, a comprehensive analysis of the correlation between pesticide detections and different land use is essential in these monitoring studies.

Regarding temporal variations, in the number of detections, the density curve presented in Fig. 3 shows the total number of detections per sampling round. Overall, all individual sampling rounds showed similar patterns, with a maximum of 5 to 8 total detections per apiary and sampling round, and a shift toward more detections during sampling rounds SR1 to SR5. A consistent decrease in higher detection values was observed across all sampling rounds. In general, a decrease in detections was observed throughout the sampling period from May to August, with a maximum variation of approximately 28 %. This can be explained because of a general decreased crop treatment. A small rebound was observed in August, largely as a consequence of an increase in the number of acetamiprid detections. In contrast to these trends, and as expected, the variability of DEET across the nine sampling rounds was markedly different, with values during the first rounds being less than 20 % of those recorded in the later rounds, reaching maximum levels in August.

Considering the temporal variations (Fig. 4) of the 31 most frequently detected compounds (those found in more than 40 sampled sampling sites) across all the nine sampling rounds, detection frequencies ranged from pendimethalin, with 107 detections in 40 sampling sites, to azoxystrobin, with 1466 detections in 237 sampling sites over the entire sampling period. It is noteworthy that acetamiprid, azoxystrobin, boscalid, tebuconazole, and fluopyram were each detected in over 100 apiaries, with approximately 1000 or more detections per compound, appearing consistently across nearly all sampling periods.

This high level of detection for these five pesticides corroborates the findings of the 2022 EU Multiannual Control Programme (MACP) (Unknown, 2025) for pesticide residues in food, as defined under Regulation (EU) No 2019/533. These five compounds were among the most frequently detected pesticides, with more than 5000 quantified results in the 110,829 samples analyzed. It is worth noting that for the majority of these compounds, there were distinct peaks in detection levels observed at specific times. The total number of detections was generally higher during the early sampling rounds, particularly in the months of May and June. This period coincides with the peak of the blooming season, a time characterized by intense foraging activity by pollinators and also increased agricultural interventions.

Of particular concern is the frequent and widespread detection of several compounds, especially insecticides, that are currently not authorized as phytosanitary products in the European Union due to the high potential for harm to human health and the environment. Notably, this includes thiacloprid, permethrin, carbendazim, chlorpyrifos, and chlorfenvinphos (Fig. 4), which were detected in more than 60 apiaries, each with over 100 individual detections. Several factors may explain their continued presence, including: emergency or grace authorizations; specific-use approvals (as in the cases of carbendazim and permethrin); recent non-renewal of authorization (as with thiacloprid); use as biocides under Directive 98/8/EC, or; in some instances, incorrect application which can encompass potential poisoning events. This latter situation is most likely the case for compounds such as chlorpyrifos and chlorfenvinphos, both organophosphates (acetylcholinesterase inhibitors), whose authorizations were withdrawn as early as 2003. This is particularly relevant in the case of chlorfenvinphos, where, despite detections coming from 40 sampling sites, over 50 % of the 282 detections originated from just 9 apiaries in Spain and three in Portugal.

The cases of imidacloprid and fipronil, due to their specific relevance for bees have been considered separately. These findings highlight the importance of having robust monitoring tools to assess whether detections of banned or restricted-use compounds are isolated incidents that are declining over time, or are indicative of a broader trend.

3.4. *Pesticide concentrations*

Approximately 65 % of the pesticides detected were present at levels below the limit of quantification (LOQ), which was set at 0.5 ng per

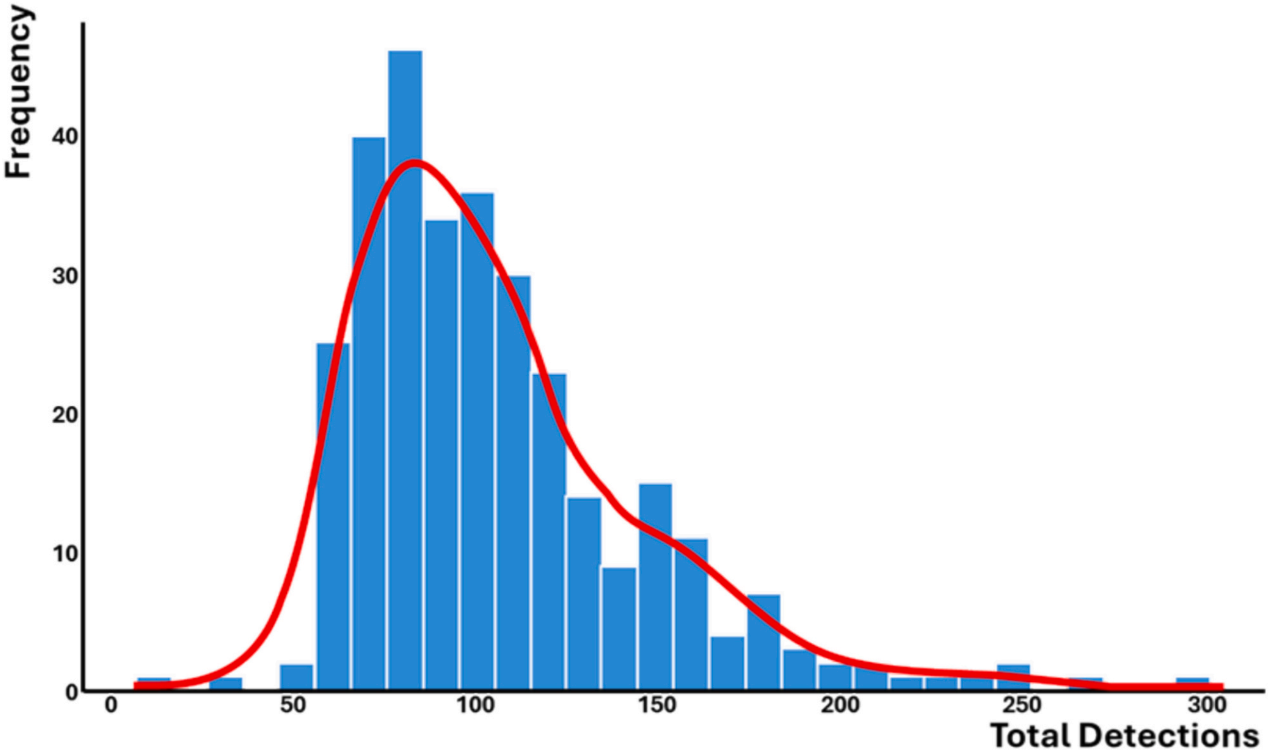

Fig. 2. Histogram of total detections grouped into 10-unit bins, with the overlaid red curve depicting the data's kernel density estimate.

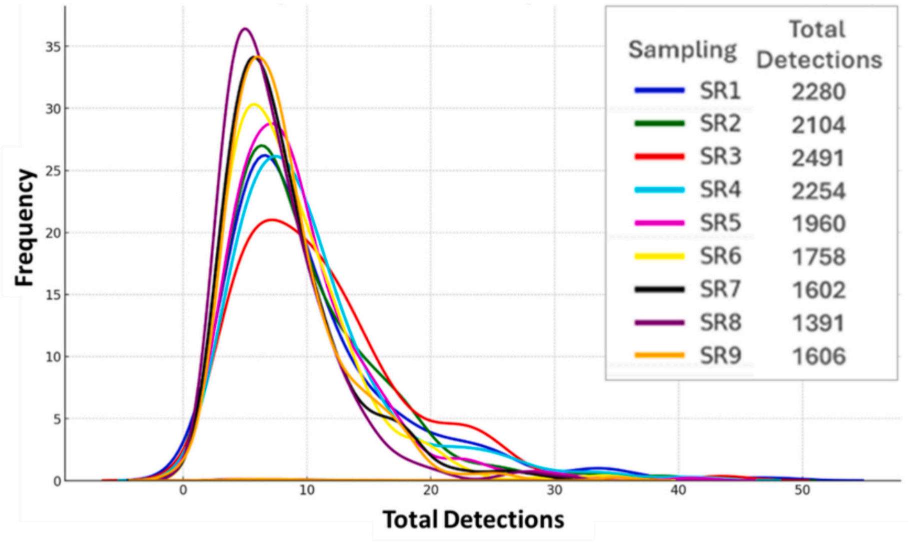

Fig. 3. Distribution of total detections by sampling round (SR1-SR9).

APIStrip for the majority of the compounds. Although below the LOQ, the compounds were confidently identified based on the presence of two transitions and an abundance ratio within 30 % of the reference standard. For reporting purposes, a default value of 0.25 ng per APIStrip was assigned to these detections. Around 25 % of the detections fell within the 0.5 to 1.0 ng range, while the remaining detections were at higher concentrations, with approximately 5 % exceeding 1.5 ng per APIStrip.

Among the most frequently detected compounds: azoxystrobin; boscalid; acetamiprid; fluopyram and tebuconazole, average concentrations ranged between 1 and 2 ng. Extending the analysis to the 31 most frequently detected pesticides (Fig. 5), average concentrations remained within a similar range for most compounds. However, notable exceptions were difenoconazole, as with DEET used as a reference with high anthropogenic use, and both of which exhibited significantly higher average concentrations, exceeding 6 ng per APIStrip.

This suggests that, while the most commonly detected pesticides are generally present at low concentrations, certain substances may be applied intensively at specific times, leading to high concentrations during particular periods. This reflects variations in application practices and usage patterns.

This is particularly relevant when considering unusually high maximum values observed in some cases, such as azoxystrobin, boscalid, and difenoconazole, with isolated detections exceeding 350 ng (Fig. 6). It is important to consider the potential for negative acute and chronic exposures to occur simultaneously, as their combination may lead to synergistic effects that are more harmful than either exposure type

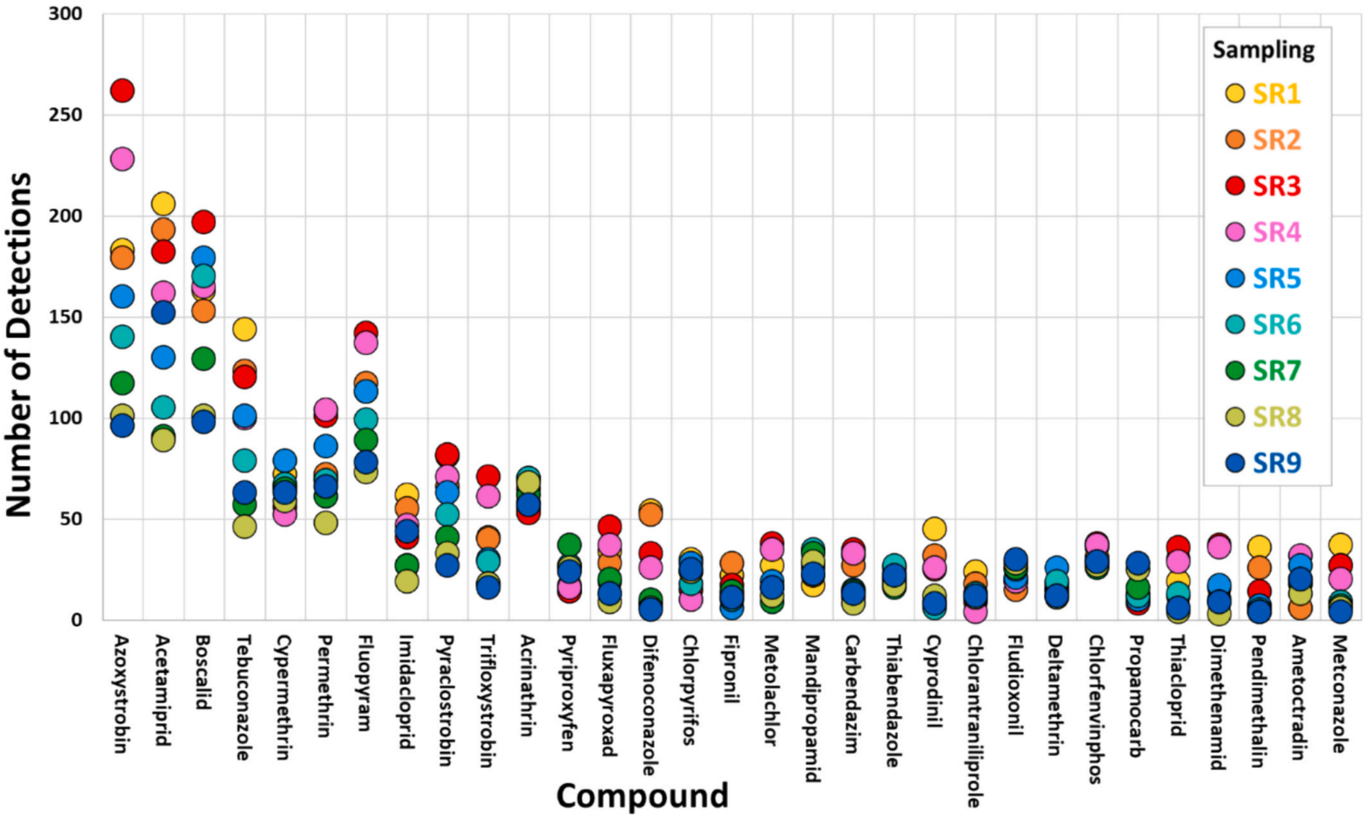

Fig. 4. Compounds detected in more than 40 sampling sites across all apiaries throughout the nine sampling rounds (SR1–SR9).

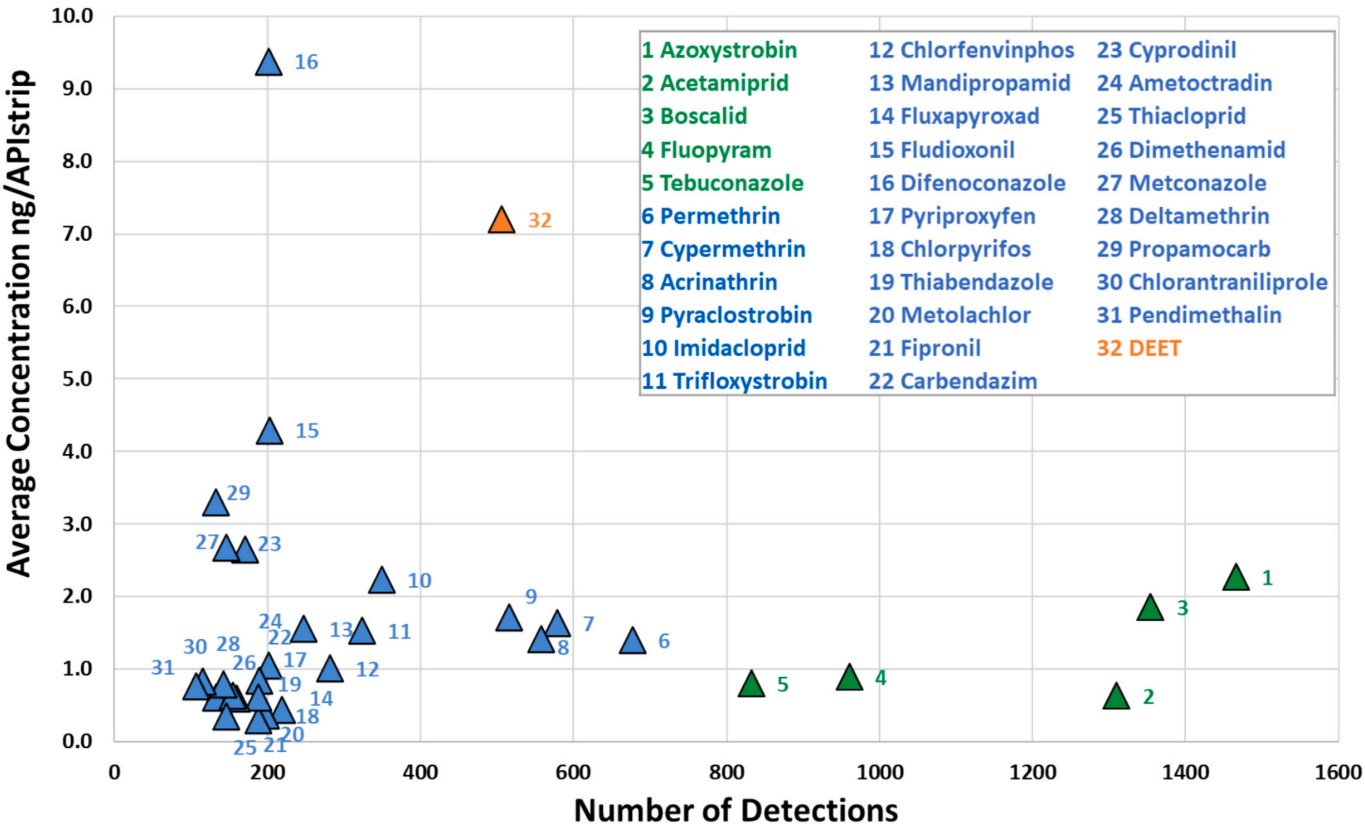

Fig. 5. Relationship between number of detections and average concentration of the 31 most frequently detected compounds. Colors indicate number of detections: highest (green), medium (blue), and the repellent (orange).

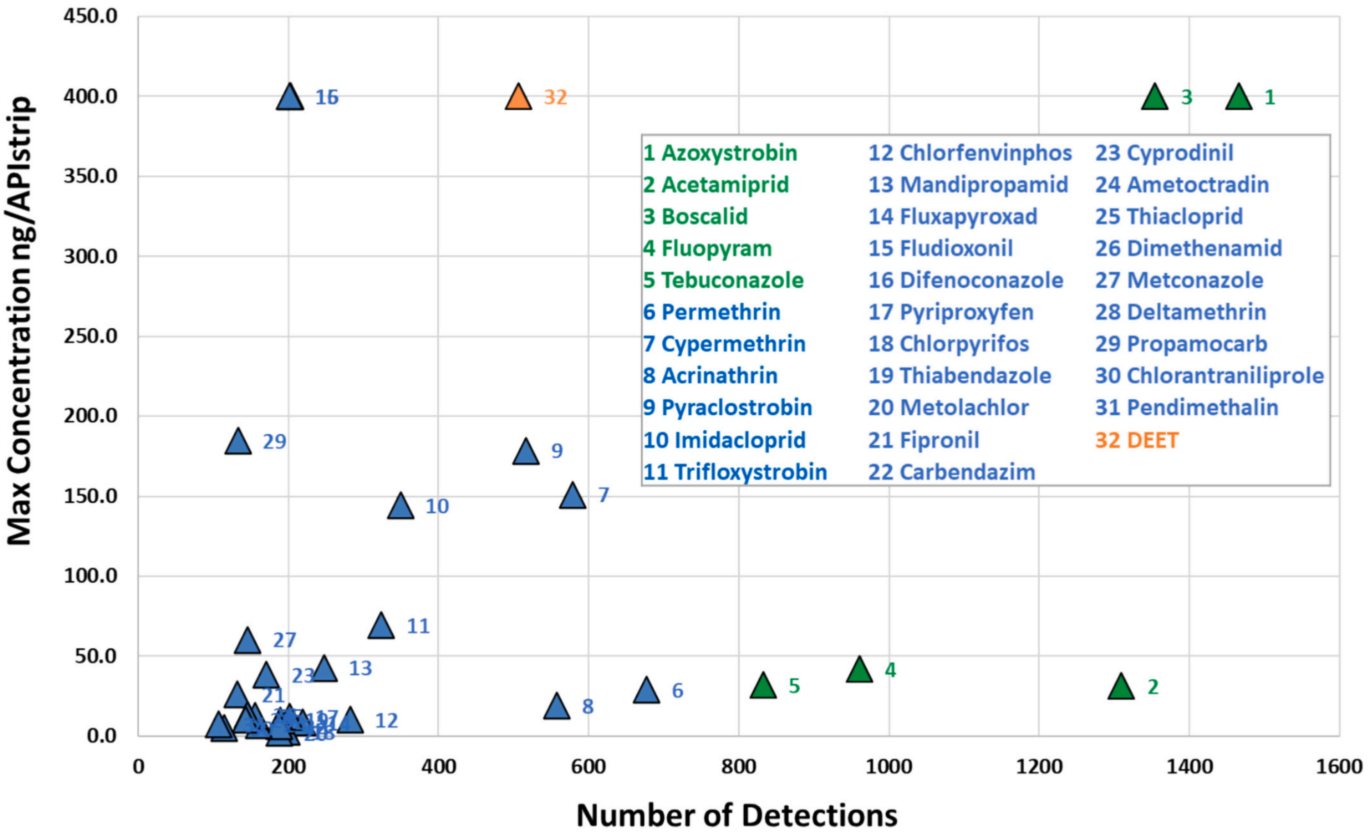

Fig. 6. Relationship between number of detections and maximum concentration of the 31 most frequently detected compounds. Colors indicate detection frequency: highest (green), medium (blue), and the repellent (orange).

alone. In the case of DEET, although it was not among the most frequently detected pesticides, the amounts measured were, on the contrary, among the highest. This demonstrates its atypical use compared to pesticides applied in agricultural practices, as well as the efficacy of the PASs used. Although it may occasionally be present at high concentrations, it is not considered relevant in this context due to its low toxicity.

3.5. *Pesticide detection, concentration and land use*

As expected, land use played a key role in both the number of detections and the concentrations of the pesticides found. Agriculturally dominant landscapes exhibited the largest number of detections, as shown in Table 1. Although agricultural areas accounted for only 54 % of the sampling sites, they contributed over 68 % of the pesticide detections, and more than 70 % of the total pesticide amount detected in the APIstrips. Normalized for the proportion of sites, This represents an index of 1.27 and 1.26 respectively. It is thus evident that agricultural land use is associated with a higher number of detections, which is also reflected in the elevated total amounts of pesticides detected. There was no significant difference between the detection index and the index for the amount of pesticides evaluated, indicating that the increase in detections was consistent with the increase in pesticide quantities. This alignment reinforces the robustness and reliability of the methodology applied in this study. Across both key metrics (detection frequency and total pesticide amount) agriculturally dominant landscapes exhibited more intensive pesticide presence, with values approximately 25 % higher in both cases relative to their land area.

In contrast, the other two land-use types contributed less to both the number of detections and the total amounts detected, with normalized indices of 0.7 or lower when considering their proportion of sampling sites. This means that at least 25 % fewer pesticides were detected and accounted for than what would be expected based on the land-use area represented in the study. There is a crossover of values for detections and total amounts between artificial and natural land uses that could represent lower applications in natural but in general at similar concentrations levels. It is interesting that, when considering only pesticides detected above the LOQ, the profiles of the 20 most frequently detected compounds are similar across all three land-use types—underscoring both the broad impact of agricultural applications and the role of spray drift. However, some compounds—such as chlorpyrifos, chlorfenvinphos, and zooxamide—were most frequently detected in dominant natural or urban areas land uses, suggesting that non-agricultural activities like gardening (Šlachta et al., 2023) and ornamental plant

Table 1
Relationship between land use and percentages of total number of detections and total amounts evaluated (in ng).

Land use	Percentage of total land use	N° of detections	Detections as a percentage of the total (Normalized index)	Total amount evaluated (ng)	Amounts as a percentage of the total (Normalized index)
Agricultural	54.0 %	11,805	68.56 % (1.27)	19,278	67.86 % (1.26)
Artificial	21.0 %	2685	15.60 % (0.74)	4021	14.14 % (0.67)
Forest / natural	25.0 %	2723	15.82 % (0.63)	5114	17.99.% (0.72)
Total	100 %	17,213	100 %	28,414	100 %

treatments can also contribute to these detections through the use of older, stored products. In particular, chlorfenvinphos—an older compound widely used for veterinary ectoparasite control (e.g., sheep dips and barn sprays)—appears most often in dominant natural land-use areas, While zooxamide is commonly associated with ornamental plant treatments, its levels are therefore expected to be much lower in agricultural land-use areas. As supposed, DEET is far more prevalent in dominant artificial and natural areas than in agricultural ones. Once again, the strong consistency between these two evaluation criteria highlights the reliability and value of the methodological approach applied in this study.

3.6. Imidacloprid and fipronil

The presence of imidacloprid and fipronil both raise significant concerns for two reasons: both compounds are banned for agricultural use within the EU; and both are highly toxic to honey bees at the ng level.

Notably, imidacloprid was frequently detected across 23 EU countries during all monitoring period. It is essential to note that the European Commission prohibited the use of imidacloprid on bee-attractive crops in 2014, with limited exceptions such as greenhouse use and at non-flowering crop stages, in accordance with Regulation (EU) No 485/2013. This was followed by a complete ban on agriculturural uses across the EU in 2018. Nonetheless, temporary derogations permitted the use of imidacloprid on specific crops until 2023, effectively ending the emergency use of these substances in the EU. Furthermore, in the case of imidacloprid as a Product-Type 18 under Regulation (EU) No 528/2012, the approval expires in 2025. In addition it remains in use as a flea treatment for domestic uses. These reasons may explain many findings of this substance during 2023.

During this study, imidacloprid was detected 349 times, in 116 sampling sites with a total quantified amount of 780 ng. The variability in the number of detections across the nine sampling rounds was small, and ranged between 20 and 50 times (Annex III). Similarly, the amounts were low, at LOD levels in 54 % of the cases, and 80 % below 1 ng per APIStrip, indicating a residual situation. Exceptionally, the peak levels exceeded 50 ng in four apiaries: two in the Netherlands, one in Spain and one in Romania. It is important to note that these four locations are not primarily agricultural land, but are instead adjacent to forests and artificial areas, suggesting that the presence of this pesticide may largely be due to non-agricultural sources.

The use of fipronil is prohibited in food products due to its potential health risks and it is banned as a veterinary drug for animals intended for human consumption. Although regulators in the European Union banned fipronil for use on crops in 2017, seeds treated with fipronil could still be used in most EU countries until 2019. However, fipronil is still authorized as a veterinary treatment for fleas, mites, and ticks in dogs and cats but is explicitly forbidden for animals within the food chain, such as chickens. Consequently, residual fipronil can persist in the environment (Perkins et al., 2024) and can be collected by honey bees during foraging. This is consistent with the very low number of detections (132 instances) recorded across all sampling rounds, as well as the low concentration levels observed in this study compared to other similarly detected compounds. Most of these concentrations were near the limit of detection (LOD), totaling 80.5 ng. The only notable level observed was at one location in Portugal, where 30 ng accumulated over the nine sampling rounds in an area classified as forest/natural. We suspect this may be due to an accidental spill or a similar isolated event, as its sulfone metabolite was not detected. However, generally, these results enable understanding of the chemicals dispersal capabilities, independent of localized authorizations and so the need of extra controls to evaluate the efficiency of the use restrictions.

3.7. The case of DDT and its isomers

DDT (Dichlorodiphenyltrichloroethane) has been banned in the European Union since 1986 for agricultural use. Its production, use, and import are strictly prohibited under EU regulations aligned with the Stockholm Convention due to its extreme environmental persistence, bioaccumulation in the food chain, and toxic effects on wildlife and human health. Its persistence and widespread historical use have resulted in ongoing contamination, with residues still detected in soil samples across Europe. Therefore, the relatively frequent detection of DDT in apiaries during this study is not surprising, considering its residual presence in the soil (Kurek et al., 2019; Kurek et al., 2025) and the capability of the plants to extract this contaminant from the soil to the pollen.

A total of 276 detections of DDT and its isomers from 68 apiaries were recorded, totaling 130 ng. The variation in detections across the sampling rounds was consistently low, with approximately 32 occurrences in each round and similarly very low concentration levels close to the LOD were observed. Notably, more significant amounts higher that 6 ng were detected in regions of a group of relatively close situated regions; in apiaries in Poland, Estonia and Romania, which featured diverse land uses of agricultural, artificial, and forest/natural respectively. Clearly, these findings cannot be directly attributed to recent pesticide use, and could be explained by isolated contaminated sites.

4. Conclusions and further studies

The employment of the APIStrip passive samplers within bee hives, coupled with beekeeper-led citizen science initiatives, has demonstrated substantial efficacy in monitoring pesticide dispersion across vast terrestrial landscapes. This methodology utilizes the natural foraging behavior of bees, which enables the APIstrips to sample a wide range of environmental conditions. The placement of these strips in bee hives significantly enhances the sensitivity and accuracy of pesticide detection, thereby providing a detailed and expansive assessment of landscape contamination.

Furthermore, this approach not only amplifies the detection capabilities for existing pesticide levels but also assists in identifying chemical threats in specific areas, thus offering a critical tool for ecological and environmental health monitoring.

The results obtained have provided an initial overview of terrestrial pesticide contamination across Europe, establishing thresholds in detections and concentrations that may serve as reference levels for future evaluations. Comparing these biomonitoring results with those obtained from different environments (water, food, etc.) can be integrated under a ‘One Health’ approach to enhance our understanding of the impact of regulations and field practices related to pesticides.

The data presented here show the presence of multiple compounds in the environment but, by themselves, cannot assess or reveal their effects on the bees collecting the samples, or on other organisms in the environment. This approach should therefore be complemented by experimental studies that help to evaluate the correlation between pesticide amounts found on the APIStrips and the residues found on honey bees. This correlation, combined with ecotoxicological data, will be essential for understanding the potential toxic effects on these pollinators, as well as their resilience thresholds and those of other organisms within the environment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180285.

CRediT authorship contribution statement

Amadeo R. Fernández-Alba: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **María Murcia-Morales:** Writing – review & editing, Methodology, Conceptualization. **José Luis Oller-Serrano:** Writing – review & editing, Conceptualization. **José Antonio Martínez:** Writing – review & editing, Conceptualization. **Jozef J.M. Van der Steen:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Robert Brodschneider:** Writing – review & editing, Methodology, Conceptualization. **Kristina Gratzer:** Writing – review & editing. **Fani Hatjina:** Resources, Conceptualization. **Norman Carreck:** Writing – review & editing, Conceptualization. **Alison Gray:** Writing – review & editing, Conceptualization. **M. Alice Pinto:** Resources, Conceptualization. **Andreia Quaresma:** Conceptualization. **Ivo Roessink:** Writing – review & editing, Methodology, Conceptualization. **Bas Buddendorf:** Writing – review & editing, Formal analysis, Conceptualization. **Marco Pietropaoli:** Resources, Conceptualization. **Konstantinos M. Kasiotis:** Writing – review & editing, Conceptualization. **Effrosyni Zafeiraki:** Conceptualization. **Evangelia Tzanetou:** Conceptualization. **Flemming Vejsnæs:** Resources, Conceptualization. **Ole Kilpinen:** Resources, Conceptualization. **Valters Brusbardis:** Resources, Conceptualization. **Dirk C. de Graaf:** Conceptualization. **Ellen Danneels:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The INSIGNIA-EU project (Preparatory action for monitoring of environmental pollution using honey bees https://www.insignia-bee.eu.) leading to this publication has received funding from the European Union's tender under EU (N° 09.200200/2021/864096/SER/ENV.D.2). This publication reflects the author's view; the European Commission is not responsible for any use that may be made of the information it contains.

The authors are deeply grateful to all the National Coordinators and beekeepers from the 27 EU countries who participated in this citizen science project. Their invaluable contributions were fundamental to achieving the results presented in this work.

Data availability

Data will be made available on request.

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