Synthesis report on agrochemical, pathogen and nutritional exposure

Deliverable D10.3

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ANSES

PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

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Summary

The aim of PoshBee Task 10.3 is to scan and synthesise the current knowledge available on pesticide exposure and its effects on bees, and the use of omics technologies for these same purposes. This deliverable D10.3 reports the results on exposure of bees to stressors recorded in field surveys. The objective of this deliverable is to contextualise the results obtained in PoshBee with the wider landscape of international research activities.

Following the population, exposure, outcomes method, we defined i) the target population, ii) the exposure (by definition of this deliverable), iii) the relevant stressors divided into three categories, and iv) the relevant survey types. We also defined a list of excluded terms from our literature review in order to better focus our review of published scientific papers.

The target population included honey bees and all pollinators. Exposure was, from the outset, the focus of this literature review. The three stressors comprised pesticides, infectious and parasitic agents (IPAs) affecting bees in general, and nutrition. The type of investigation described by a paper was particularly important as we only targeted surveys (as opposed to studies with an experimental set up) so as to better compare with the fieldwork implemented in PoshBee. Finally, we excluded from our literature review some terms such as genetic modified organisms and antibiotics as these were not included in PoshBee protocols.

Only one paper reported three stressors (IPAs, pesticides and nutrition) surveyed at the same time and on the same population. Colony losses was the end-point surveyed. Nutrition quality was evaluated through a measure of the total protein content of beebread samples. The presence of DWV in autumn was positively correlated with colony losses, while the following year colony losses were significantly related to the presence of pesticides in honey bees. In both years, colony mortality rates were positively related to the percentage of agricultural land surrounding apiaries, supporting the importance of intensive land use in causing poor honey bee health.

The systematic quantitative review identified 36 scientific papers reporting field surveys that investigated two factors or stressors. Only one paper involved three species of bee (bumble bees, mason bees and honey bees, the same species as for PoshBee), but the survey looked at the interaction of only two stressors, IPAs and pesticides. Among IPAs, the most frequently mentioned IPA influencing the end-point of surveys was *Nosema ceranae* (in 12 studies). The combination of stressors linked to exposure to IPAs together or with other stressors (pesticide exposure, nutritional quality) was also underlined in several surveys. PoshBee's own field survey involved 384 bumble bee colonies, 288 mason bee nests and 384 honey bee colonies (3 colonies in each of 128 sites except for Ireland and United Kingdom for mason bees). PoshBee's target population was at the high end of all studies surveyed (median among published studies: 83.5 honey bee colonies; from 11 (minimum) to 11 500 (maximum) colonies were included in the surveys). The same conclusion can be drawn with the number of IPAs employed in a study (11 IPAs in PoshBee, whereas the median was 7.7 from the literature review) and the number of pesticides studies (550 in PoshBee, whereas the median was 87.3 from the literature review).

From this literature survey we can already state that the PoshBee fieldwork was unique in terms of the number of bee species involved (three bee species), the number of stressors surveyed (three stressors), the number of colonies/nests involved (384 per target bee species) and the geographical extent of the site network (in PoshBee, fieldwork was set up at a continent level including country specific diversity). The employment of 'omics technologies made PoshBee's field survey even more of a novel outlier as they have been used only once before in a study limited to a restricted area. The statistical analysis of PoshBee results will allow ranking of stressors affecting bee health. To our knowledge, this ranking has never been done in any field survey involving three stress factors.

1. Introduction

An aim of PoshBee Task 10.3 is to scan and synthesize the current knowledge available in scientific literature on pesticide exposure and effects on bees, and the use of 'omics technologies to do so. This deliverable reports only the results of the literature surveys on exposure of bees to stressors recorded in field surveys. Please refer to PoshBee deliverable D10.4 for information on the effects on bees and PoshBee deliverable D10.5 for information on 'omics in determining the impact of stressors (particularly pesticides) on bees. The objective of this work was not to produce a stock take of knowledge available at the date of writing. Rather, the aim was to put the results obtained in PoshBee into the wide landscape of international research activities.

2. Material and Methods

The literature search covered the period from 2000 to 2021; given that the targeted field studies were not numerous, the aim was to include as many field surveys as possible in this systematic quantitative review (Pickering and Byrne, 2014). Following the population, exposure, outcomes method, we defined i) the target population, ii) exposure (as the focus of this deliverable), iii) the relevant stressors divided into three categories, and iv) the relevant survey type. We also defined a list of terms which we excluded from our literature review in order to better target our search of the scientific literature. The target population included honey bees and all pollinators. The stressors for which we sought literature included pesticides (including all categories such as fungicides, herbicides and insecticides), infectious and parasitic agents (IPAs) affecting bees in general, and nutrition, using in particular the chemical prism of proteins, lipids or sugars. The type of investigation was particularly important as we only targeted surveys as opposed to studies with an experimental set up so as to more easily compare literature results with the results of fieldwork implemented in PoshBee. Finally, we excluded from the literature review some terms such as genetic modified organisms and antibiotics as these were not included in the PoshBee protocols. Seven search strings were developed (Figure 1) based on keywords. These terms were searched in the titles, abstracts and keywords of papers in Scopus and CAB abstract databases. A similar protocol was used in literature searches of the effects of exposure (D10.4) and omics (D10.5) to enable cross-comparison of results with this deliverable. Search strings were, however, adapted for D10.4 (literature review of pesticide exposure) to be of relevance for the studies implemented in PoshBee.

#1 Population

Honeybee OR apis* OR beehive OR bee OR pollinator

#2 Exposure

Exposure OR "risk assessment"

#3 Pesticides

Pesticide OR fungicide OR herbicide OR insecticide OR agrochemical OR chemical OR metals OR metabolite OR neonicotinoid

#4 Pathogens

Pathogen* OR parasite* OR varroa* OR disease* OR virus* OR mite OR health OR pests OR bacteria

#5 Nutrition

Nutrition OR probiotic OR protein OR lipids OR sugar OR "fat body" OR pollen OR nutrient* OR diet* OR "floral resources" OR nectar OR "bee bread" OR beeswax

#6 Field survey

Monitoring OR agroecosystems OR “agricultural environment” OR crop OR field OR culture OR area OR nature OR domain OR zone OR land OR territory

#7 Excluded

GMO OR antibiotic OR “immune response” OR laboratory OR “electromagnetic field” OR "Dead bees"

Figure 1. Strings developed to search for scientific papers on the assessment of bee exposure to stressors in field surveys in different databases. Strings characterized the population under study (#1), the type of exposure (#2), pesticides (#3); pathogens (#4), nutrition (#5), surveys (#6) and excluded papers (#7). In green: keywords common to three systematic quantitative reviews (on exposure, on effects and on omics). In red: keywords specific to the exposure systematic quantitative review.

Following abstract examination, duplicates and papers out of scope were excluded from the analysis. Many papers reported studies involving human interventions that could subsequently be qualified as experiments, and were likewise removed. Studies in which honey production as an endpoint was used as this end point was not surveyed in Poshbee, and literature reviews were also excluded. After this step, all papers were read and some were again excluded on the basis of the removal criterion ‘experimental’ (i.e. with the involvement of experimental procedures). References of all articles were scrutinized in order to feed the final database with articles in the scope of our literature search. At the end of these processes, 37 scientific articles were selected (Figure 2).

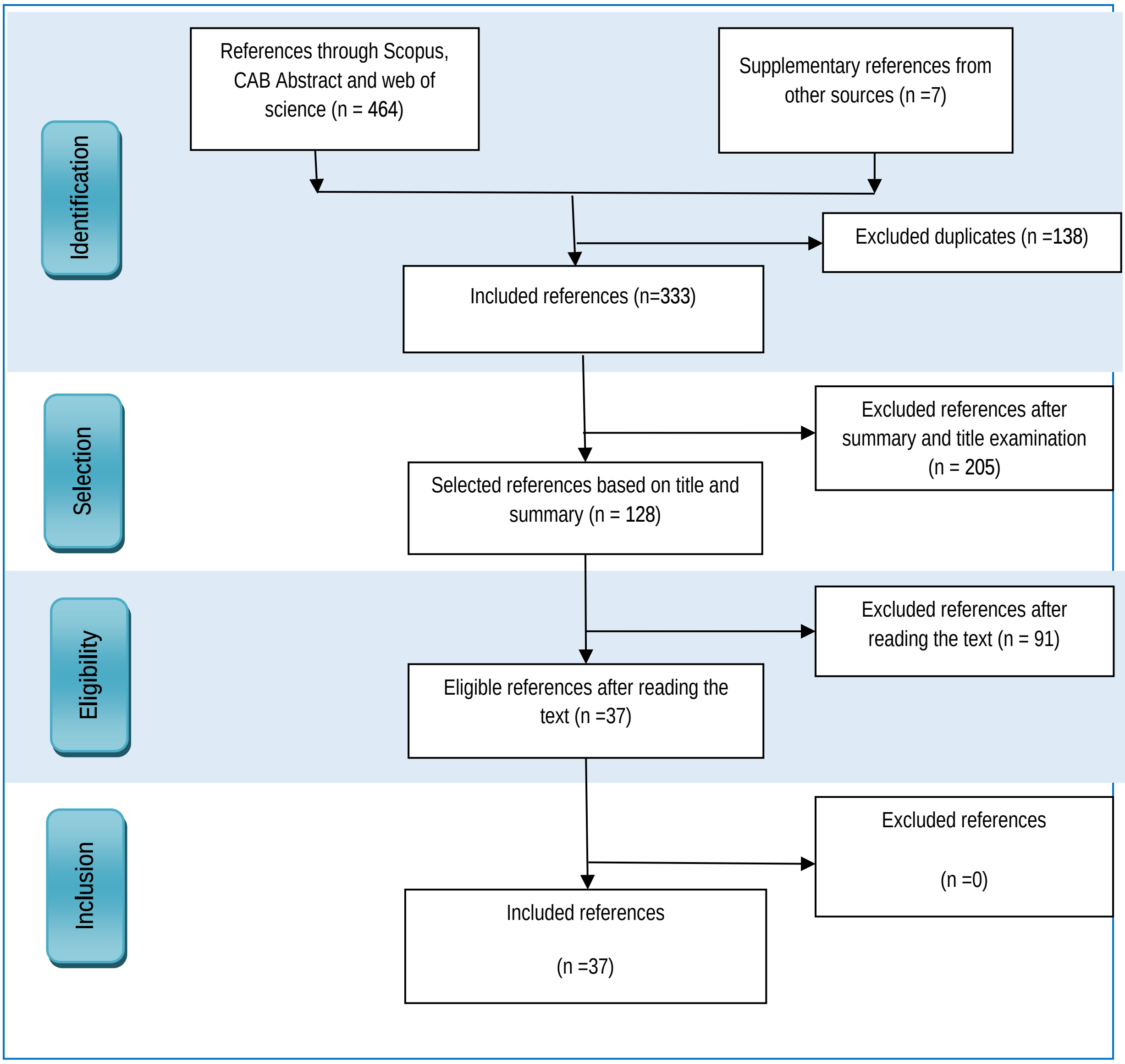

Figure 2: Prisma flow resulting in the 37 selected papers assessing bee exposure to stressors in field studies (surveys).

3. Results

3.2 Field surveys with three stressors surveyed

Of the 37 papers making the final list, only one paper reported three stressors (IPAs, pesticides and nutrition) surveyed at the same time and on the same population (Porrini et al., 2016). Moreover, stressors were only surveyed on honey bee colonies. In this paper, undertaken in Italy, a monitoring network was established in 2009 and 2010 including 130 apiaries located across the country. IPAs (n=11) were detected in bees and pesticides (n=128) were detected in bees, beebread and beeswax. Colony losses was the end-point surveyed. Nutrition quality was evaluated through the measure of the total protein content of beebread samples. The prevalence of *N. ceranae* ranged, on average, from 47–69% in 2009 and from 30–60% in 2010, with strong seasonal variation. Virus prevalence was higher in 2010 than in 2009. Coumaphos, propamocarb, tau-fluvalinate and flumethrin were the most found compounds through multicompound analysis. Statistical analyses did not allow the ranking of stressors related to the risks posed to honey bee colony survival. However, the presence of DWV in autumn was positively correlated with colony losses while, in the following year, colony losses were significantly related to the presence of pesticides in honey bees. In both years, colony mortality rates were positively related to the percentage of agricultural land surrounding apiaries, supporting the importance of land use for honey bee health.

3.2 Field surveys with two stressors surveyed

The systematic quantitative review identified 36 scientific papers reporting field surveys that investigated two stress factors. The field surveys described were mostly located in Europe (n=21), predominantly in Spain (n=7), Germany (n=4) and France (n=2). Ten field surveys were conducted in the USA and three in Canada. In Latin America, Uruguay hosted three field surveys. The African and Asian continents were represented with one survey each (in Japan and in Kenya). All the papers were published between 2008 and 2021 (maximum number of publications per year is 5 in 2010, 2014, 2015 and 2016). Most of the investigations (n=25) were designed to survey honey bee colonies in natural conditions (i.e., without specific settings). However in some cases (n=11), the surveys were set up in particular environments to specifically study unexplained honey bee (Kimura et al., 2014) or colony losses (Pistorius et al., 2009).

The pesticide-IPA association was the most studied pair of stress factors (n=33). Nutritional quality was assessed together with pathogens twice and in one study together with pesticides. Apart from these four studies which assessed the nutritional value of the environment, 20 surveys made some analysis to assess the environment (foraging area), the vast majority (n=15) being palynological analyses of pollen pellets or beebread. The surveys involved from 20 to 11 500 colonies (median 83.5 colonies), located in regions of a few kilometres radius to vast areas. Honey bee colonies were the only subject of study in all studies except for one that also included bumble bee colonies and mason bees (Rolke et al., 2016b). Most of the surveys looked at colony health (measures of honey bee population, n=12). The other major measured end-points were colony losses (n=8), winter colony losses (n=6) and colony disorders (n=4). In one survey, colony morbidity, exposomics (i.e., chemical features found via gas chromatography-time of flight mass spectrometry [GC-TOF]), pollen nutritional quality, and pesticide exposure were together used as end-points (Figure 3). The statistics linked some stressors to these end-points. Conversely, significant non-links (i.e. factors that had statistically no influence on end-points) were also highlighted in the publications and are reported in our literature review.

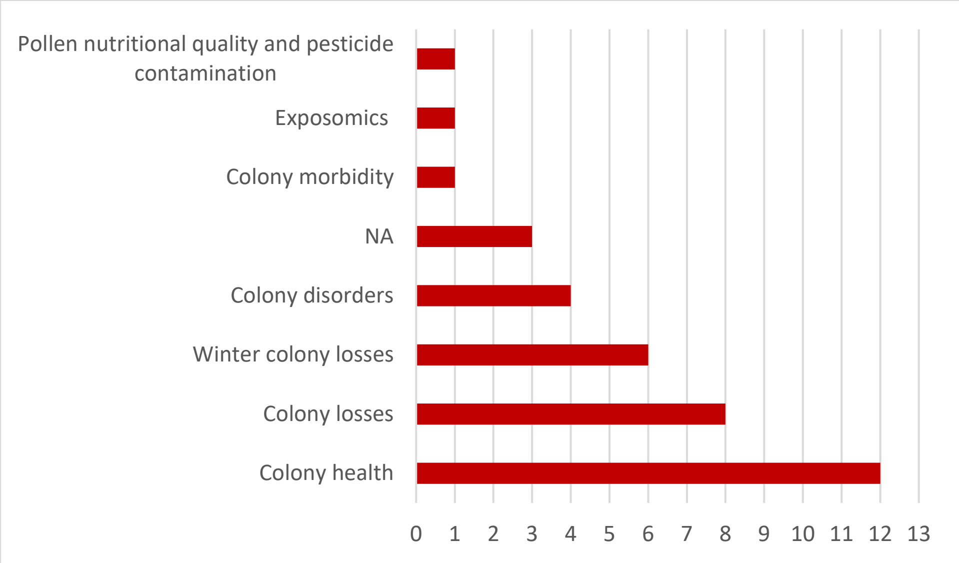

Figure 3: Type of end-points reported in the 36 selected papers. NA: not applicable i.e no end-point were defined.

The factors that were reported to influence end-points were divided in twelve categories (Figure 4). Some IPAs were specifically mentioned in surveys (American foulbrood, Kashmir bee virus, *A. woodi*, deformed wing virus, *V. destructor*, *N. ceranae*). Colony condition in general and starvation in particular were reported as factors negatively influencing the end-points. 'Queen' refers to any problem linked to queen failure or misbehaviour. When the authors specify a type of flower (eucalyptus and rape/mustard) influencing the end-point, it was reported under 'Flowers'. The features surrounding study sites (crops, agricultural areas, nutritional stress, regional importance, environmental condition) of honey bee colonies were mentioned as influencing end-points and referred to in 'Environment'. Under the 'Pesticides' category, all studies were gathered in which the results found a specific active ingredient, a group of ingredients (i.e. fungicides) or treated crops influencing the end-points.

IPAs were the main factors influencing the various end-points in the field surveys (n=19), alone or in combination with other factors (with pesticides or several IPAs together), followed by pesticide exposure (n=17) and environment (n=7). Pesticide exposure was found to be responsible for negatively acting on the end-points in 16 field surveys. Fungicides (included in 'Pesticides' in Figure 4) were specifically mentioned to be linked with colony disorder or pathogen prevalence in studies in Belgium (SimonDelso et al., 2014), Italy (Martinello et al., 2017) and in the US (Traynor et al., 2016; Traynor et al., 2021).

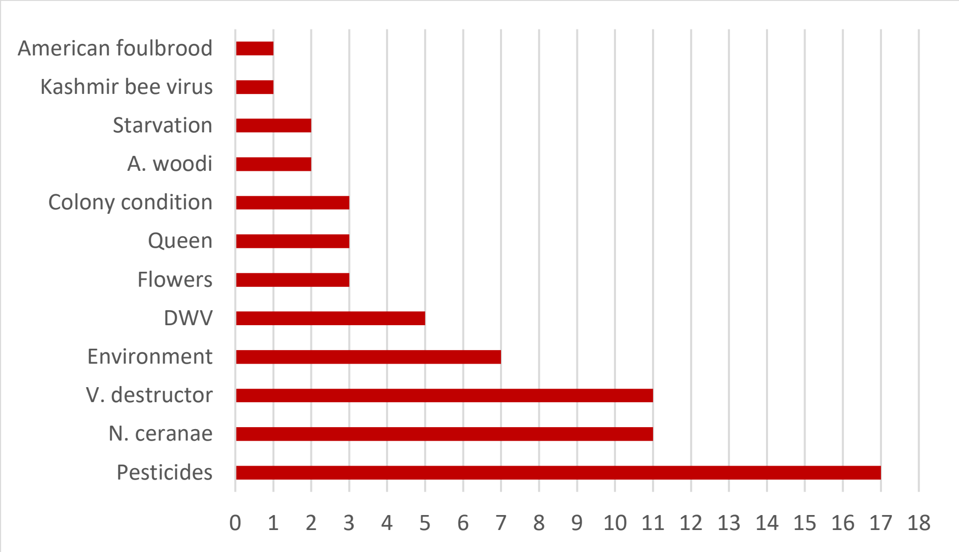

Figure 4: Factors influencing the various end-points reported in the 36 scientific papers (see the text for category description).

Among IPAs, the factor influencing the most end-points in the surveys was *Nosema ceranae.* This microsporidian parasite was the risk factor most strongly associated with colony losses or depopulation in 11 studies. In 11 field surveys, varroa mite treatment and/or loads were the strongest factors for explaining honey bee colony losses in Europe, the US and Uruguay. During blueberry production in the US, the varroa mite was the main factor responsible for poor colony health of migratory hives. In addition, the presence of DWV (a virus linked to varroa mites) in autumn was related with surprisingly high significance ($P = 0.00001$) with winter losses in Germany (Genersch et al., 2010). In France, in more than 76% of apiaries, one or several severe diseases known for being able to totally destroy colonies were present (Faucon et al., 2015). Interestingly, results obtained in Kenya indicated that parasites and pathogens were not impacting honey bee health, at least in terms of colony size, suggesting that environmental factors (climate, landscape ecology) may play a key role in mediating this host-parasite interaction (Muli et al., 2014).

The combination of stressors linked to exposure to IPAs together or with other stressors (pesticide exposure, nutritional quality) was also underlined in several surveys. In Germany, varroa mites plus viruses were the most probable cause of colony failure (Rolke et al., 2016a). In Greece, imidacloprid, viruses and *N. ceranae* synergistically played the most important role in colony losses (Bacandritsos et al., 2010). The same authors also concluded that nutritional stress and *Nosema* spp. infection had a severe impact on colony strength, with consequences in both the short and long-term (Bacandritsos et al., 2010). In Spain, the combination of the two pathogens *N. ceranae* and *V. destructor* could augment the risk of colony death in infected colonies (Bernal et al., 2011; Higes et al., 2009).

Three field studies, whose results were published in two sets of papers (six papers in total), focused on IPA exposure and the link with honey bee colony health on one hand (Clermont et al., 2015; Rolke et al., 2016a; Vanengelsdorp et al., 2012) and on pesticide residues exposure and the link with colony health on the other hand (Beyer et al., 2018; Rolke et al., 2016b; Traynor et al., 2016). It is detrimental to our understanding that, from a global analysis, the two sets of data concerning pathogens and pesticides were not statistically analysed together.

4. Discussion

The analysis of the 36 papers on bee exposure to at least two stress factors in the field has shown that the PoshBee fieldwork is a unique piece of research. As expected, the systematic quantitative review only yielded a small number of papers (n=36). Indeed, these types of field studies are time-consuming and require considerable investments in time, money and human resources. Therefore, although they are valuable in producing close to realistic conditions and they provide much information, they are not often set up.

The PoshBee field work included three stressors on three species of bees. In our literature review, we found only one paper dealing with three stressors, but this looked only at honey bee colonies (Porrini et al., 2016). Only one paper involved three species of bees (bumble bees, mason bees and honey bees, the same species as in PoshBee), but the survey looked at the interaction of two stressors, IPAs and pesticides; not three. As noted earlier (Benuszak et al., 2017; Havard et al., 2019), there is a lack of data in this field of enquiry generated from Africa and Asia (only one survey for each continent in our study). The field surveys reporting two stress factors on honey bee colonies were done mostly in Europe (n=13) and Northern America (n=9). Some published studies were conducted at the country-level, and as a result only those done in the USA (Traynor et al., 2016; Vanengelsdorp et al., 2012) approached the scale of PoshBee, which was done at the continental scale, in eight European countries distributed across Europe's main biogeographic zones. The PoshBee field survey involved 384 bumble bee colonies, 288 mason bee nests and 384 honey bee colonies (3 colonies in each of the 128 sites). This magnitude of population under study was in the upper range of the sample size of studies from the literature review (the median was 83.5 honey bee colonies; from 11 (minimum) to 11 500 (maximum) colonies were included in the surveys). The same conclusion can be reached with the number of IPAs detected (11 IPAS in PoshBee, whereas the median was 7.8 from the literature review; from 0 (minimum) to 18 (maximum) IPAs were included in the surveys) and the number of pesticides detected (550 in PoshBee, whereas the median was 87.3 from the literature review with a minimum of 1 and a maximum of 258 pesticides searched in the surveys).

Regarding the end-points, the PoshBee field survey looked at the health of bumble bees, mason bees and honey bees (measuring bee population), as also reported in 12 papers (we also included in this category n=4 papers with colony disorders as the end-point). Only one paper included exposomics (i.e. chemical features found via gas chromatography-time of flight mass spectrometry [GC-TOF]) as an end-point (Broadrup et al., 2019), which was also included in the PoshBee survey. Concerning the factors that influenced the health of bee populations, it is unfortunately too early to draw firm conclusions from the PoshBee work to include in this report. These conclusions will be provided by PoshBee Work Package 2 partners and will be compared to the results from the literature review (the pesticides linked to colony disorders in 16 surveys) at a later stage.

However, from this literature survey we can already state that the PoshBee fieldwork is unique, given the number of bee species involved (three bee species), the number of stressors surveyed (three stressors), the number of colonies and nests involved (384 for honey bee and bumble bee colonies and 288 for mason bee nests) and the geographical extent of the site network (in PoshBee, fieldwork was set up at a continent level, including country specific diversity). Assessment of 'omics gives the PoshBee field survey additional value, as this approach has only been used once before in a restricted area (Broadrup et al., 2019). The statistical analysis of PoshBee results will enable ranking of the stressors affecting bee health. To our knowledge, this ranking has never been done for any field survey involving three stress factors.

5. Acknowledgments

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