Report on training

Deliverable D11.7

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

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Preface

In **Task 11.5 Training,** PoshBee aims to build bee health capacity among early career researchers, veterinarians and other specialists via targeted training. To achieve this, project partner UMONS organised and held a European Bee Course via a two-day workshop. This dedicated training school was held in Mons, Belgium on 12th and 13th April 2022 and was open to all stakeholders inside and outside of PoshBee. In particular, there was a strong emphasis on targeting veterinarians, early career researchers (master students, PhD students and postdocs) and beekeepers. Upon review of the applications, a total of 15 applicants were accepted and invited to participate. The training school's agenda had a firm focus on developing practical bee-related skills and special attention was paid to bumble bees and solitary bees. During the workshop, researchers presented the new analysis techniques developed within PoshBee and disseminated the main project results, providing insights on their importance for future research and better beekeeping and pollination in Europe.

Summary

As part of the project's engagement plan for relevant EU stakeholders and effective dissemination of project results, PoshBee organised a dedicated training school on developing bee-related research skills. During this workshop, researchers from UMONS presented new practical analysis techniques developed within the framework of PoshBee. The thematic focus of the training put emphasis on bumble bees and solitary bees and included a variety of laboratory and field experiments. Supplementary materials such as training videos were developed and distributed to describe the different sampling methods and further support the workshop activities.

A total of 15 applicants (see Annex 2 for more information) were accepted and invited to participate in the training school. Due to health issues, only 9 trainees attended the event. The training school was held over two days, both of which included four presentations and three experiments. The training days started at 9am and concluded around 5.30pm. After the workshop concluded, an evaluation was conducted in order to analyse the quality of the workshop and identify areas of improvement for future training events.

This deliverable provides a comprehensive overview of the training content, its agenda, participants, presentations, and evaluation.

1. Introduction

The PoshBee training school took place on 12th and 13th April 2022 in Mons, Belgium. Its goal was to build bee health capacity among early career researchers, veterinarians and other specialists via targeted training. In order to reach as wide an audience as possible, PoshBee started promoting the training school a month and a half before the event took place. A promotional poster was designed (see Figure 1) and a dedicated news article was published on PoshBee's website in March 2022, elaborating on the workshop's agenda and including instructions on how to apply for it. Furthermore, PoshBee's social media channels were used to spread awareness, promote and report on the event (see Figure 2).

Figure 1: Training promotional poster

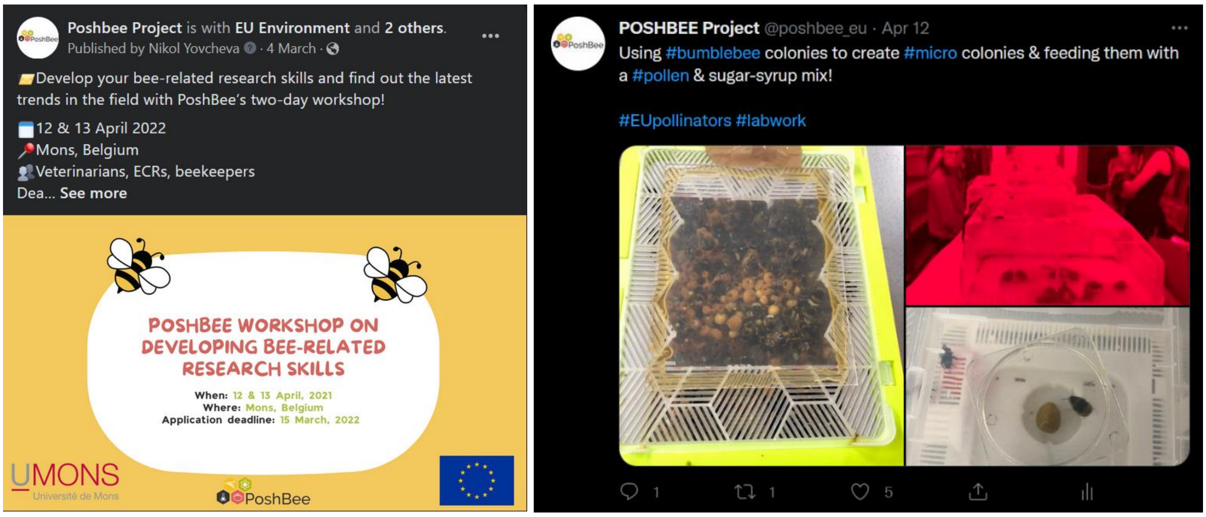


Figure 2: Social media posts promoting the workshop

The application deadline was initially set for the 15th of March, but was extended by one week in order to allow all interested parties to apply. Upon review of the applications, a total of 15 applicants were accepted and invited to participate (with 9 trainees attending the two-day-long training school), most of whom were early career researchers from more than 5 countries. The applications were reviewed and accepted by UMONS based on their background, profile, and motivation to join the course.

To introduce PoshBee's EU stakeholders to the new analysis techniques and experiments developed within the project, the school trainers – Prof. Denis Michez and PhD students Alexandre Barraud, Antoine Gekière, and Kimberly Przybyla from UMONS – devised presentations from the field of Poshbee research. In addition, further information on wild bees generated by the UMONS lab focused on bumble bee and solitary bee studies was included. Following the background presentations, the team presented several dedicated laboratory and field practice experiments. This allowed the participants to get hands-on experience in working with wild bees. The workshop also presented the main results of the PoshBee project to date and provided insights on their importance for future research and bee health in Europe.

Finally, the trainees were provided with training materials to help them acquire new bee-related research skills. For example, twelve short instructional PoshBee training videos were available to trainees during the workshop (see **our PoshBee YouTube channel** for more information). These videos demonstrated the PoshBee protocols in practice and contributed to an easier knowledge transfer.

2. Content of the training

The training school was held over two days, both of which included four presentations and three experiments. The training days started at 9am and concluded around 5.30pm. This section of the deliverable presents a short and concise summary of the sessions of the workshop. To see the detailed agenda, please consult Annex 1.

Figure 3: Start of the PoshBee training school

2.1. Bumble bee ecology, diversity and population trends

In the workshop's first presentation, Professor Denis Michez gave a comprehensive overview of bumble bee ecology, diversity and population trends. He started from the origins of the species, showing its distribution around the world and the diversity of its various species (around 250). Denis also discussed the variations in bumble bee morphology, floral choices and habitats. Finally, he presented a review on the drivers of bumble bee decline such as habitat loss, loss of floral resources, pesticides, parasites, invasive species and climate change.

Figure 4: Opening presentation by Professor Denis Michez

2.2. Bumble bee ecotoxicology

Alexandre Barraud gave a presentation about bumblebee ecotoxicology. He discussed how the toxic effects of compounds on bumble bees are studied in order to assess their consequences at a populational scale. The presentation included a concise history of how pollution evolved to what we know today and which of its aspects are particularly toxic to bees. Afterwards, Alexandre went on to discuss toxicity risk assessments and which parameters can help measure risks. For example, such parameters include mortality, reproduction, flying ability, nutritional intake, olfaction, and immunity.

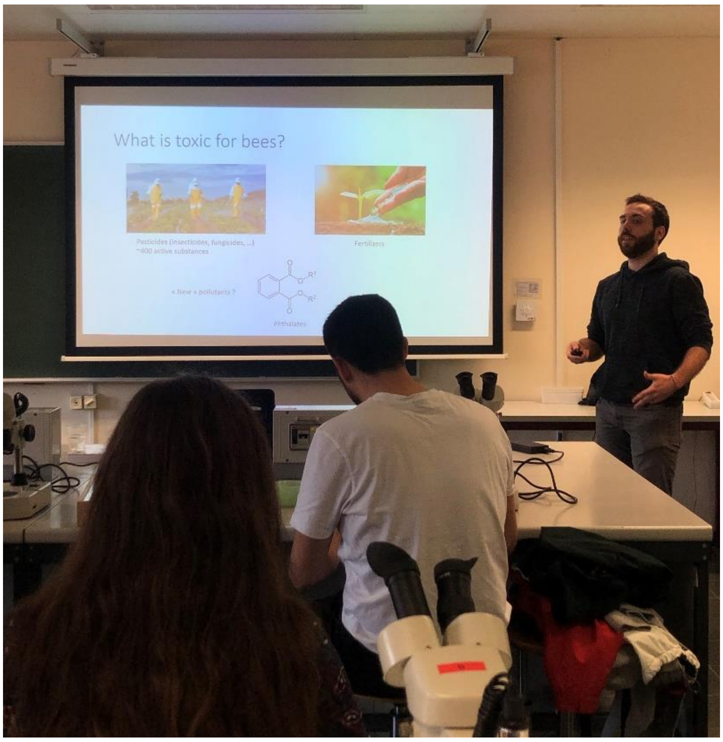


Figure 5: Alexandre Barraud presenting a review on eco-toxicology of bumble bees.

2.3. Study of bumble bees in laboratory conditions

Kimberly Przybyla delivered a presentation on bumble bee breeding. During this presentation, she elaborated on her experience of field and semi-field experiments in which the model species *Bombus terrestris* was used, and presented the materials used in the experiments. She addressed the laboratory conditions of the experiments and what their findings were. Kimberly explained the conclusions reached on the life cycle of bumble bees, as well as on the new insights for wild bee nesting methods. Finally, she referred to one of PoshBee's training videos, called "Effects of agrochemical-nutrition interactions on bee health in the laboratory".

Figure 6: Laboratory experiment on toxin exposure

2.4. End point parameters: Bumble bees

The afternoon of day 1 was dedicated to demonstrating experiments and ended with a presentation on end point parameters for the study of bumblevbees. In this presentation, trainers elaborated on the end point parameters at different levels: colony and micro-colony, specimen and molecular level. Denis Michez informed the group of pollen efficacy and brood development at colony and micro-colony level. Antoine Gekière described parasite monitoring at a specimen level and the presentation also discussed the fertility, fat body analyses and phenotype at specimen level. Finally, to present related molecular level research, researchers referred to PoshBee's training video called "Analysis of haemolymph with MALDI BeeTyping ®".

Figure 7: Experiments and practice work with bumble bees

The following experiments / methods were presented:
- Monitoring bumble bee micro-colonies
- Characterisation of phenotype based on wing size and shape
- Exposure to heat stress
- Haemolymph collection

Micro-colonies monitoring

Microcolonies consist of a group of workers housed together in cages and fed with syrup and pollen (see Figure 8). Microcolonies are queenless, but in the absence of a queen, a worker usually develops its dominance and start laying eggs (Vanderplanck et al. 2014). All the workers take care of the nest and after a few weeks, the first haploid males emerge.

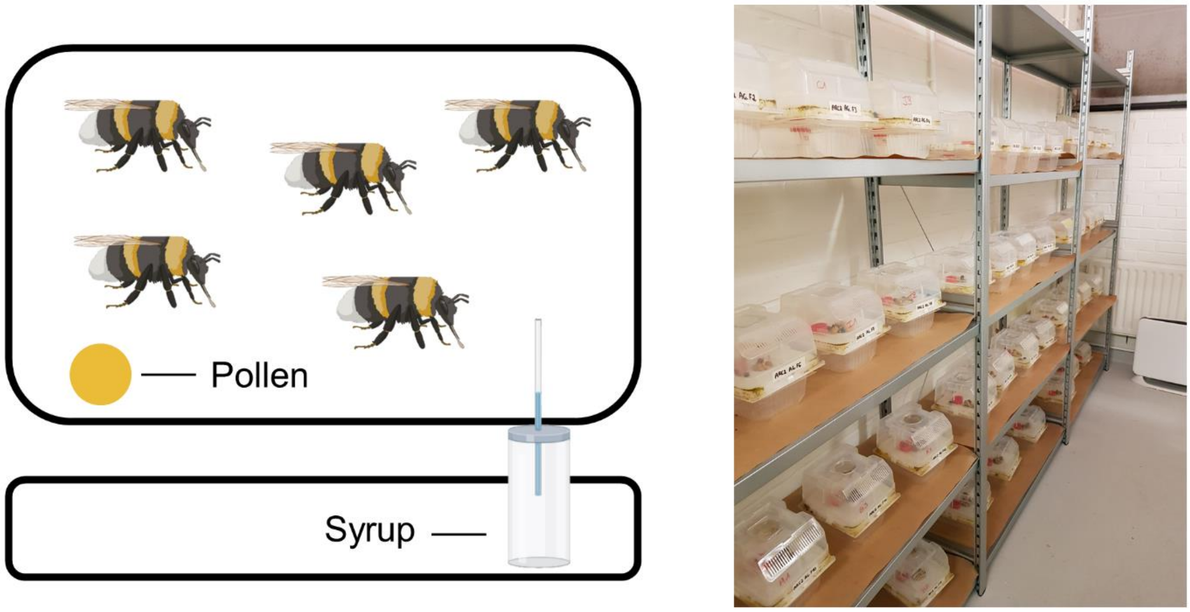


Figure 8. Left. Microcolony design. **Right.** Microcolonies in a rearing room. The left figure was created using BioRender (https://biorender.com/).

Microcolonies are useful to assess the impact of various treatments (e.g., pesticides in pollen, infected workers, etc.) on bumble bee nest development and individuals. For instance, one can monitor the food collection over the experiment and weigh the offspring production at the end of the experiment among the different treatments (Fig. 9). One can also monitor parasite infection among workers and evaluate several physiological parameters (Klinger et al. 2019). However, caution must be paid when extrapolating the results to queenright colonies (Van Oystaeyen et al. 2021).

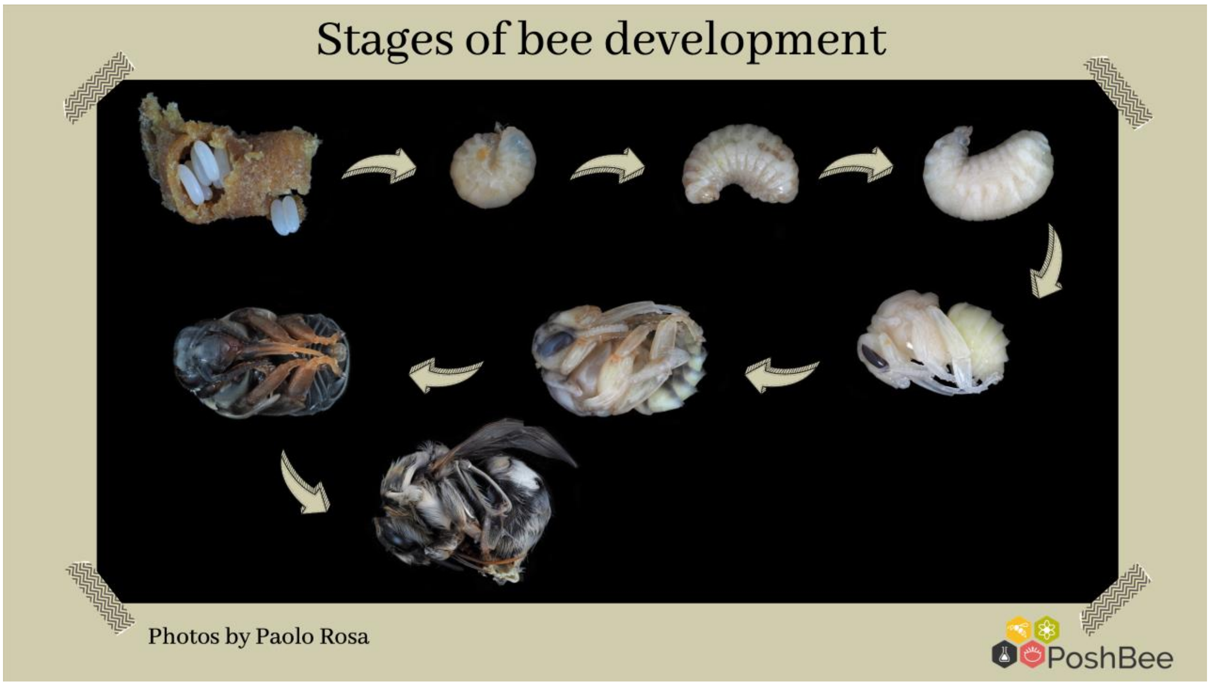
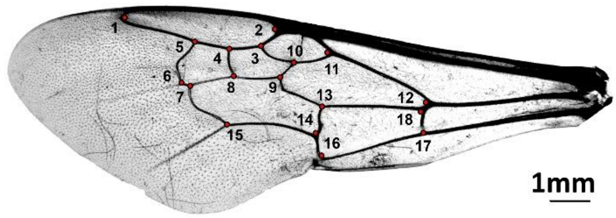


Figure 9. Development of a bumble bee from egg to imago.

Characterisation of phenotype based on wing shape

Shape and size are key biological traits that enable the evaluation of how phenotypic variation across individuals develops as they undergo different environmental pressures (Gerard et al. 2018). In bees, such traits are particularly important as they are directly linked to both physiological abilities and foraging efficiency. Thus, when studying the impacts of various stressors on bees, it is relevant to consider phenotypic variation. Phenotypic variation can be efficiently assessed using both left and right forewings since this structure is in 2D (i.e., easy digitalisation), and the same protocol can be used regardless of taxonomic group. It is crucial to study such variations in individuals that have been exposed to the stressors during larval development. Briefly, to do so under laboratory conditions one can collect F1 individuals and remove their wings. Wings are then individually placed on slides, digitised, landmarks are placed (see Figure 10) and finally morphometric analyses are run. Three metrics are generally used: (i) centroid size, (ii) wing shape and (iii) fluctuating asymmetry. While (i) and (ii) are used as proxies for the canalisation of the phenotype, (iii) is a proxy for developmental stability.



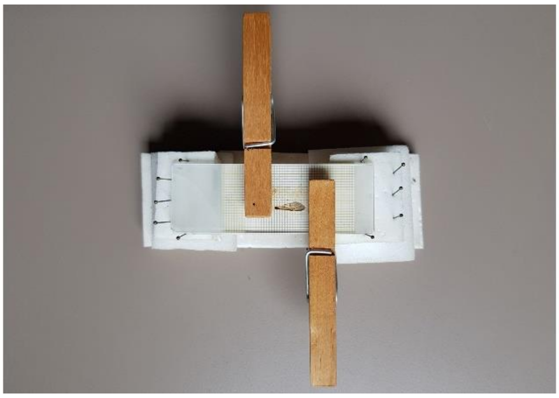


Figure 10. Left. A bumble bee left forewing with its 18 landmarks (Vanderplanck et al. 2021). **Right.** Wing flattened between a glass and a cover slide squeezed with clothespins.

Exposure to heat stress

Exposure to multiple stressors is an important field of research in the Poshbee project. Consequently, we provided training in a method to evaluate resistance of bumble bee and solitary bees to heat stress. This method determines the heat resistance of these individuals under heatwave temperature conditions (40°C) (Martinet et al. 2021). In this method the time before heat stupor is monitored (THS). The longer a specimen resists exposure to 40°C, the more resistant it is. Specifically, bumble bees and solitary bees were put in plastic containers and placed inside a set up that was heated directly at 40°C to simulate a heatwave. The individuals were left in the set up until they reached the stage called heat stupor. Beforehand, individuals under thermal stress were shown so that trainees could recognize this state. At this physiological stage the bee is on its back, unable to get back on its legs and has muscular spasms in the legs. Once the bee has reached this stage, we measured the time spent inside the set up. This metric enables comparative analysis of resistance across species. The manipulation for *Bombus terrestris* could not be carried out in full because of the high resistance to thermal stress of this species (>8 hours at 40°C). For O*smia*, the experiment was fully implemented since they have lower resistance (1-2) hours.

Haemolymph collection

The trainees were able to practice haemolymph collection from the two bee species mentioned above. They used a capillary and tube device developed by the BioPark Archamps team. To extract the haemolymph, bees were first put inside a restraint tube with a pusher to avoid being stung and the glass capillary of the device was inserted between the 2nd and 3rd tergite of the bee (see figure 11). The haemolymph enters the tube by capillary effect. It is then put inside a pre-coated Eppendorf to avoid melanisation and stored at -20°c for further proteomic analysis. Only a few microliters are required for analysis. As such this method can work for several species, but not for very small species.

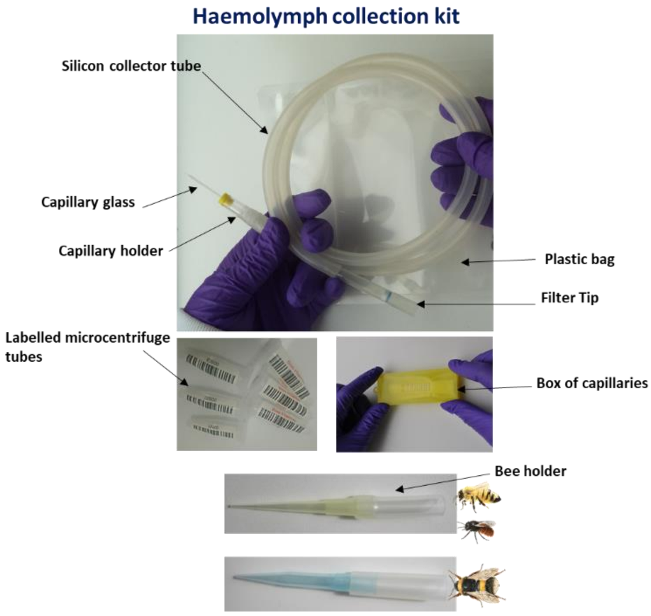


Figure 11. Haemolymph collection kit

2.5. Systematics and diversity of wild bees

The second day of the training school opened with a presentation on the systematics and diversity of wild bees. It started with an overview of their development through time, while also presenting the diversity of their morphology, behaviour and sociality. The presentation then specifically focused on the diversity of European bees and their distribution on the continent. Finally, the talk ended with an overview of bee taxonomy and systematics and an overview of their decline.

Figure 12: Opening presentation by Professor Denis Michez on day 2

2.6. Ecotoxicology of solitary bees

This talk started with an overview of the routes through which solitary bees are exposed to anthropogenic pollutants. It then presented the existing OECD guidelines on chemical testing, which show that honey bees remain the main model species used in risk assessment and that there are no directly usable guidelines and protocols for managed solitary bees,. However, the presentation ended on a positive note, explaining that new methods are under development to assess the chemical pollution risk on solitary bees.

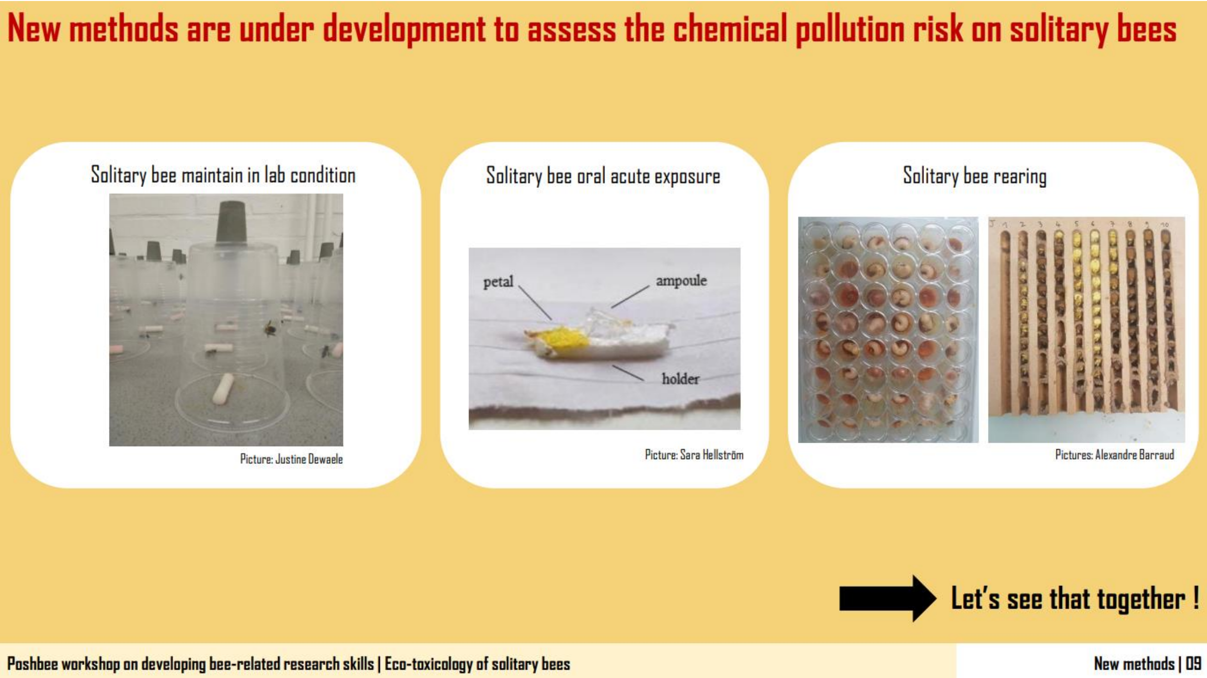


Figure 13: Final slide of the ecotoxicology solitary bee presentation

2.7. Study of solitary bees in laboratory conditions

This presentation was delivered by Antoine Gekière and introduced a number of *Osmia* species, such as *Osmia cornuta* and *Osmia bicornis*. It had a particular focus on rearing of *Osmia* species. Finally, Antoine referred to PoshBee's training video called "[Methods to assess the health status of the solitary bee *Osmia bicornis*]".

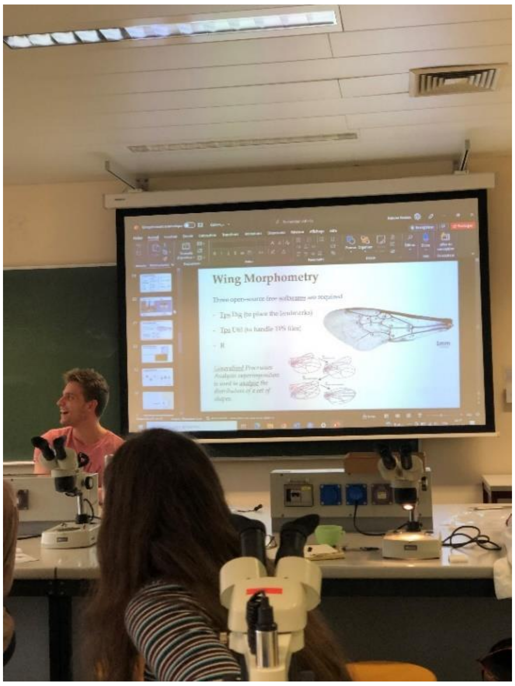


Figure 14: Antoine Gekière presenting

2.8. End point parameters: Solitary bees

The training school ended with a talk on the end point parameters for solitary bees. Trainers presented experience, materials and protocols to test the sensitivity of solitary bees to pesticides. They elaborated on the end point parameters at specimen and molecular level. First, they discussed the development, fat body analyses, and phenotype at specimen level (similar to the methods developed for bumble bees). They referenced and introduced PoshBee's training video called "Analysis of haemolymph with MALDI BeeTyping ®"in relation to the end point parameters at the molecular level.

Protocol to test adult solitary bee to pesticides

Day 1 – Acclimatization min. 8H

- Minimum of 30 individual (+ 20% if possible) bees per condition are weighed, placed under numbered cups and provided with syrup 50/50 *ad libitum* via soaked cotton capillaries.
- The cups are placed in a breeding room in the dark/in red light for an acclimatization period of at least 8 hours (until the next day).
- The distribution of cages for each treatment is carried out to avoid differences in mass.

Day 2 - Starvation 4H (morning)

- The capillaries are removed from the cups.
- Bees are left in the breeding room without syrup for at least 4 hours.
- During this time, individual eppendorfs are prepared with 50 µL of treatment solution.

Example:

ID	mass indiv (g)	Treatment	Dose (µg/g b.w)	dose indiv. (µg)	Concentration in 40 µL (µg/µL)	Volume sufloxaflor (0.01 mg/mL) (µL)	Volume syrup (µL)
1	0.322	ctrl	0	0	0	0	50
2	0.252	LD50	0.563	=mass indiv*dose	=dose indiv/40	= Concentration in 40 µL *50/0.01	= 50 – Volume Sufloxaflor

- Eppendorfs are vortexed to ensure homogenization of the solution.

Day 2 - Oral exposure 3H max (afternoon)

- 40µL of solution from the eppendorf are placed in spectrophotometry cuvettes. These are installed under the cups.
- 3 cuvettes with 40µL of syrup are weighed and arranged in the same way but without the bee. They will be reweighed at the end of exposure and will serve as a control for evaporation.
- Every 30 minutes, consumption is controlled.
- As soon as a bee has consumed the solution, the cuvette is removed and replaced with capillaries soaked in 50/50 syrup.
- Bees that have not consumed syrup after 4 hours are considered non-feeders and are not included in the experiment.

Days 3/4/5/6 – Observations (96H max)

- Mortality and sub-lethal effects are controlled 16H after the end of exposure.
- A new check is carried out at +24H, and at +48H.
  - If the mortality of all conditions (considered separately) does not exceed 10% after 48H, the experiment stops.
  - If the mortality of the treatments exceeds 10% after 48 hours while that of the control specimens remains below 10%, the experiment is continued. A check is then carried out at +72H and +96H.
- The experiment stops in any case after max. 96H.

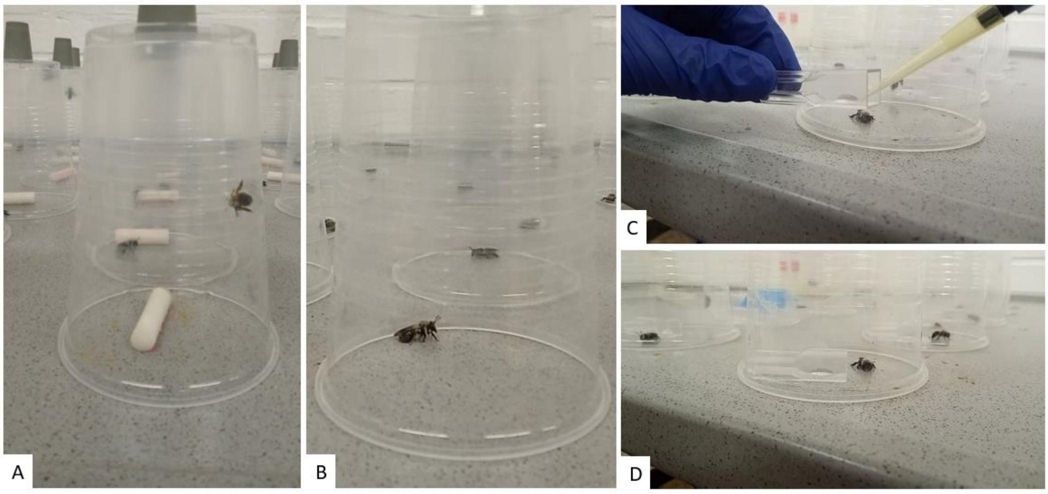


Figure 15: experimental setup. A) Acclimatation period, B) starvation period, C) deposit of the 40µL droplet, and D) exposure.

3. Evaluation

At the end of the training school an evaluation was conducted in order to analyse and estimate the quality of the workshop. The eleven trainees were asked to fill out a survey with four questions in Mentimeter:

1. How do you rate the quality of the workshop preparation and organisation?
2. How do you rate the quality and content of the presentations?
3. How do you rate the practical aspects of the training?
4. If you have to associate the workshop with one word, what would this be?

The majority of the trainees rated the preparation and organisation and the practical aspects of the training as very good. Additionally, all the trainees rated the quality and content of the presentations as very good. Finally, all participants described the workshop with positive words (see Annex 3 for full evaluation).

4. References

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Annex 1: Workshop agenda

Day 1	
09:00-10:15	Welcome at the laboratory of Zoology, goals and framework of the project
	Round table, presentation of participants (5 minutes each trainees / trainers)
10:15-10:30	**Coffee break**
10:30-12:00	Bumble bee Ecology and diversity (including the study of pinned specimens in collection)
	Bumble bee eco-toxicology
	Bumble bee breeding, experiment in field, semi-field and laboratory condition (including demonstration of the material)
12:00-13:00	**Lunch Break**
13:00-15:00	Laboratory experiment on toxin exposure at colony (queen less and queen right) level
	LD50 experiment at specimen level (acute and chronic exposure)
	Exposure to multiple stressors (pesticide, parasite, temperature, nutrition)
15:00-15:15	**Coffee break**
15:15-17:30	Study of end point parameters at molecular levels (proteomics -> collection of haemolymph), individual (-> fertility, fat body, size and wing shape) and colony level (-> pollen efficacy, mortality, brood development): description of the methods and collection of samples
19:00-21:00	**Social event at Mons (Bar Citizen)**

Day 2	
09:00-10:00	Diversity and Ecology of European bees (including the study of pinned specimens in collection)
10:00-10:15	**Coffee break**
10:15-12:15	Eco-toxicology of solitary bees
	Solitary bee breeding, experiment in field, semi-field and laboratory condition
	Osmia manipulation (nest, egg transfer)
12:15-13:15	**Lunch Break**
13:00-15:30	Sampling of solitary bees in the field (*Andrena vaga, Andrena flavipes*)
	LD50 experiment on solitary bees (acute exposure)
	Heat stress experiment on solitary bees
15:30-17:00	End point parameters at molecular (haemolymph) and individual level (body size, wing size and shape): description of the methods and collection of samples
17:00	**End of training**

Annex 2: List of participants (Trainees & Trainers)

Trainees

- Anissa Azaz Chebieb, MSc student at UMONS, Belgium
- Lucie Baltz, PhD student from the Martin-Luther-University Halle-Wittenberg, Germany
- Alice Deiss, MSc student at Lund University, Sweden
- Remi Devorsine, MSc student at UMONS, Belgium
- Ambre Dormal, MSc biology student at UMONS, Belgium
- Liam Kendall, PostDoc at Lund University, Sweden
- Klara Leander Oh, PhD student from Wageningen University & Research, the Netherlands
- Patrycja Pluta, PhD student from the Martin-Luther-University Halle-Wittenberg, Germany
- Artur Sarmento, research fellow at the University of Coimbra, Portugal

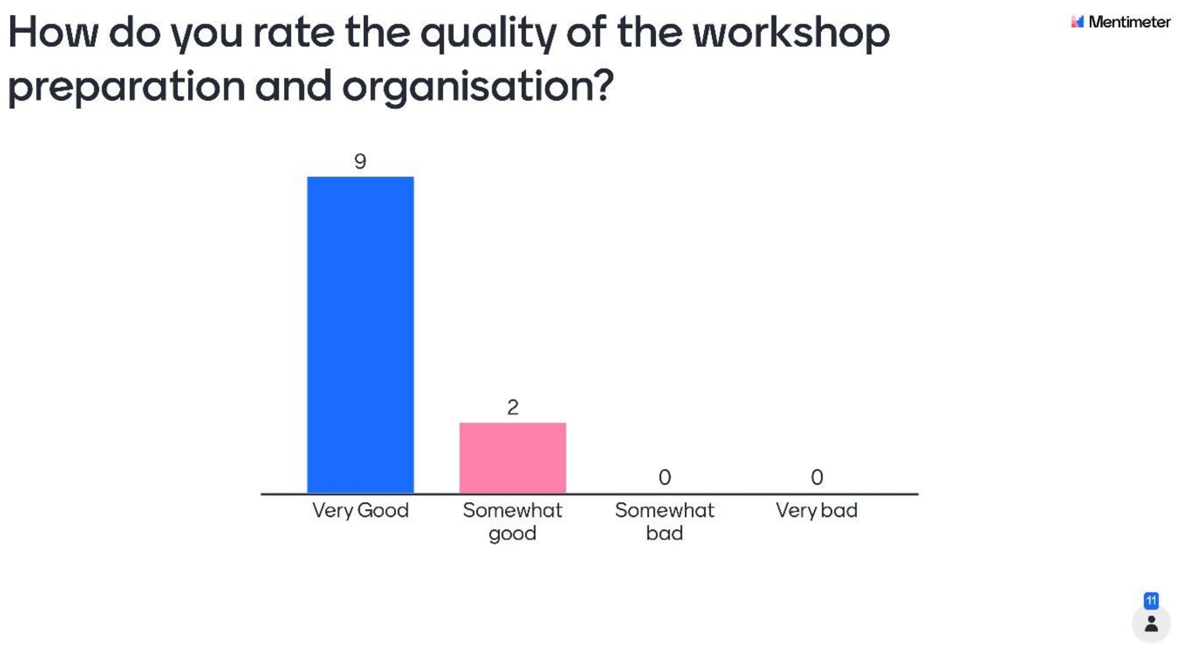
Trainers

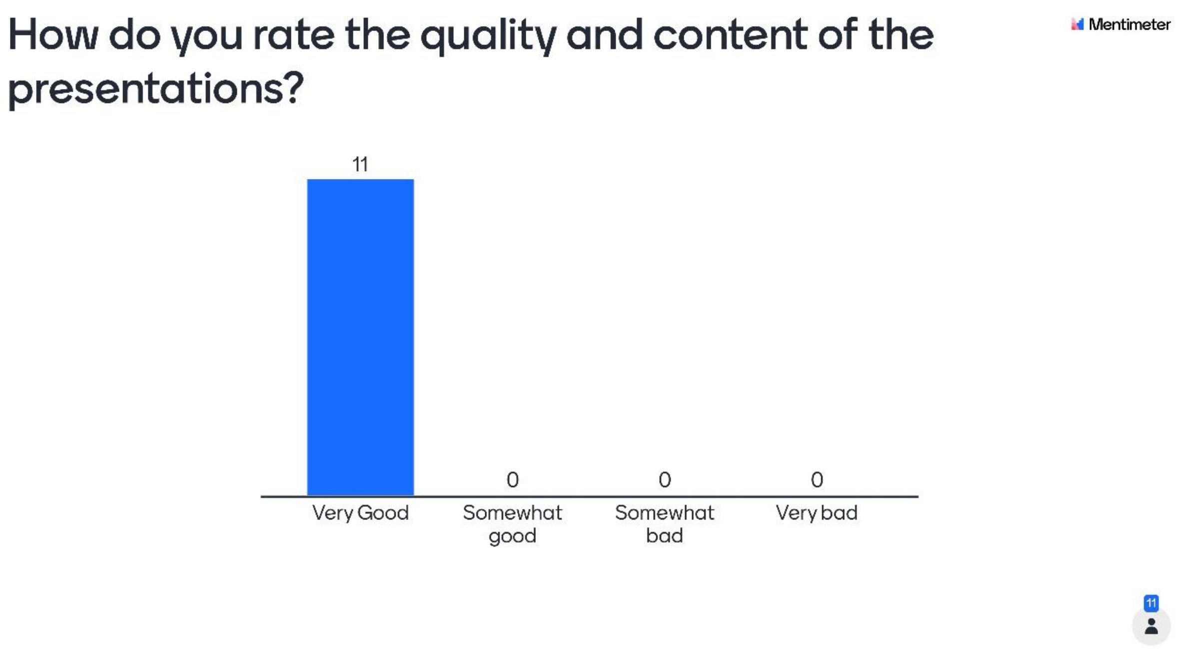
- Prof. Denis Michez, Senior researcher at UMONS, Belgium and PoshBee WP5 leader
- Alexandre Barraud, PhD student at UMONS, Belgium
- Antoine Gekière, PhD student at UMONS, Belgium
- Kimberly Przybyla, PhD student at UMONS, Belgium

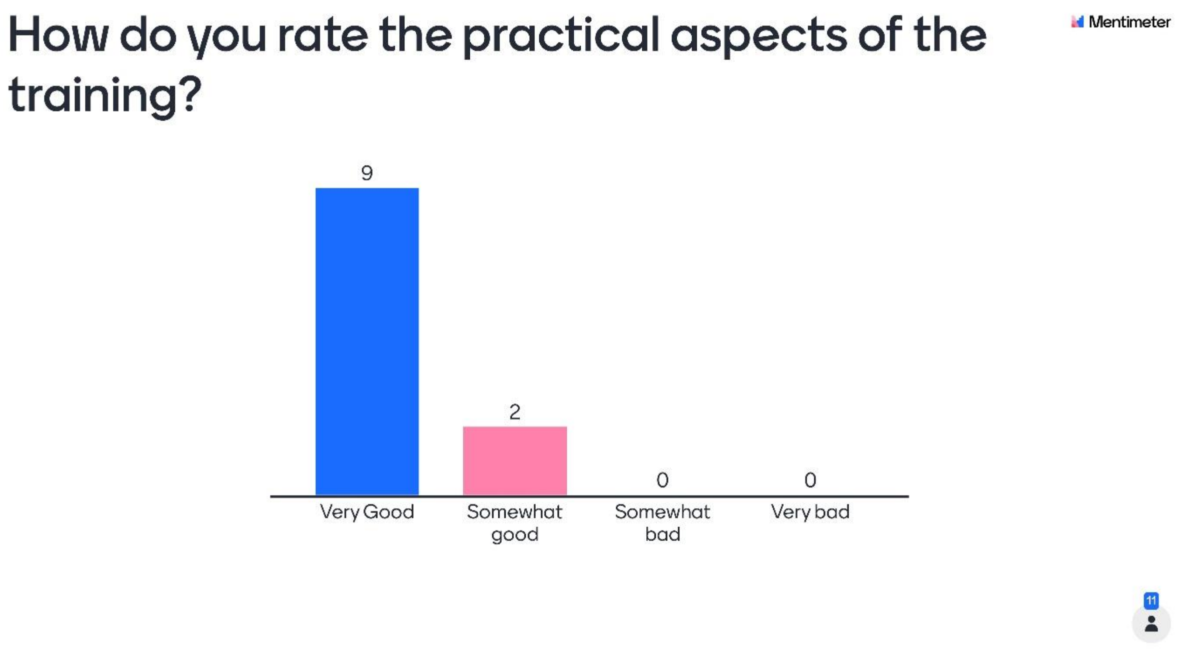
Communication & dissemination

- Teodor Metodiev, senior science communication expert at Pensoft, Bulgaria and PoshBee WP11 leader

Annex 3: Evaluation of the event






How do you rate the practical aspects of the training?
Mentimeter
9
2
0
0
Very Good
Somewhat good
Somewhat bad
Very bad
11


If you have to associate the workshop with one word, what would this be?
Mentimeter
relaxed
diverse
interactive
experience
interesting
complete
applied
informative
bees
well-prepaered
concise
insightful
friendly
chill
beeutiful
9

Annex 4: Training school presentations

A list of the presentations delivered during the two-day-long training school can be found here. For further information, all the presentations can be downloaded from the internal communication platform (ICP) of the PoshBee website (in folder WP11 → Training school at Mons (April 2020)).

- Agenda First Day
- Bumble bee ecology, diversity and population trends
- Bumble bee ecotoxicology
- Bumble bee breeding and laboratory conditions
- End point parameters: Bumble bees
- Agenda Second Day
- Systematics and diversity of wild bees
- Eco-toxicology of solitary bees
- Studying *Osmia* spp.
- End point parameters: Solitary bee