Investigating the effects of metal exposure on individual honeybees and colonies

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BACKGROUND

- Honeybees are crucial food crop pollinators
- Their health and populations are under threat from various sources (disease, pesticides, environmental factors, metals)
- The foraging activity of bees exposes them to environmental contaminants
- Anthropogenic activity has increased metal availability in the environment, such as Cu in pesticides, Cd in fertilisers, Pb, Hg and Ni in fossil fuel emissions
- Metals are well known for interacting with and causing issues in the neurological systems of organisms
- Metals available in the environment are a health stressor that plays a role in colony fitness
- Honeybees function as a superorganism where each individual fulfils a role important to the overall function and health of the colony
- Laboratory caged bee experiments show Cd and Pb impair immunity and detoxification systems, cause higher mortality rates and induce behaviour changes in honeybees[1,2]

AIMS

1. Determine where metals accumulate within the honeybee brain
2. Determine how metal accumulation interferes with gene expression
3. Examine the changes in colony health from long term exposure to sub-lethal metal doses

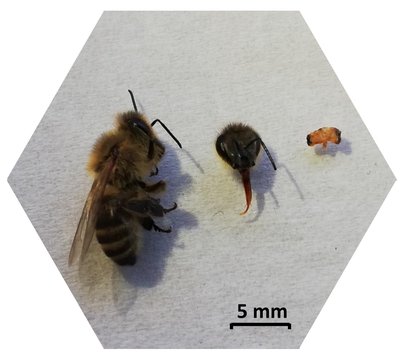

Figure 1. Honeybee body, head and brain

METHODOLOGY

This research will be carried out in two sections; laboratory-based trials and field-based trials. Details for both of these sections are given in the following table (**Table 1**).

Table 1. Details of the laboratory and field-based trials

	Laboratory-based bee trials	Field-based bee trials
Benefits	Variables controlled, better homogeneity of individuals	Natural environment, longer duration
Metal doses	Copper, Cadmium, Lead, Zinc, Manganese	Copper or Cadmium (will be based on preliminary trials)
Description	• Groups of newly emerged bees (n = 30) will be treated with varying concentrations of different metals (treated), or not treated (control) • Bees will be kept at 30°C and 70% RH for 10 days • Data will be gathered during the trials with various analyses carried out at the conclusion of each trial (see Data and Analyses)	• Treatment and control hives (n = 8 each) will be placed at an appropriate location (low environmental metal load) • Treated hives will be dosed with metals through protein and sucrose supplements • Three year trial duration, bees from each individual hive will be taken monthly (over warm seasons) to analyse
Data	Daily food/water consumption, daily mortality	Monthly colony health (brood mortality, parasite load, honey production, hive weight), forager activity, hive temperature and humidity

Analyses (both trials)	• Physical bee parameters (size, weight, deformities)
	• Transcriptomic analysis of the brain to determine variance in gene expression
	• Metal load in the head, thorax and abdomen using ICP-MS
	  • Nitric acid digestion, see Grainger *et al*. (2021)[3]
	• 2-dimensional mapping of metals in the brain using LA-ICP-MS (preliminary analysis – **Figure 2**)
	  • Bee brain fixed with rapid plunge freezing, slice brain into 30 µm sections using a cryostat
	  • 2-dimensional elemental analysis using LA-ICP-MS (30 µm spot size, 120 µm scan speed, 20 Hz repetition rate, 1.64 J/cm^2 pulse energy)	

RESULTS & OUTPUT AIMS

- This research will give a holistic overview of the effect of anthropogenic metals on the health of the honeybee
- Preliminary results show the ability of the LA-ICP-MS method to provide clear 2-dimensional mapping of elements in the honeybee brain, showing regions of high and low metal accumulation (**Figure 2**)
- Changes in cellular processes (gene expression) will be correlated to metal accumulation within brain tissues and the physical effect on individual honeybees
- Three year long field trials with treated hives will extrapolate the chronic effects seen in individual bees to the long term, sub-lethal effects on the superorganism (honeybee colony)
- This research will provide further understanding on the effect of anthropogenic activity on honeybees, and indirectly, its risk to global food production

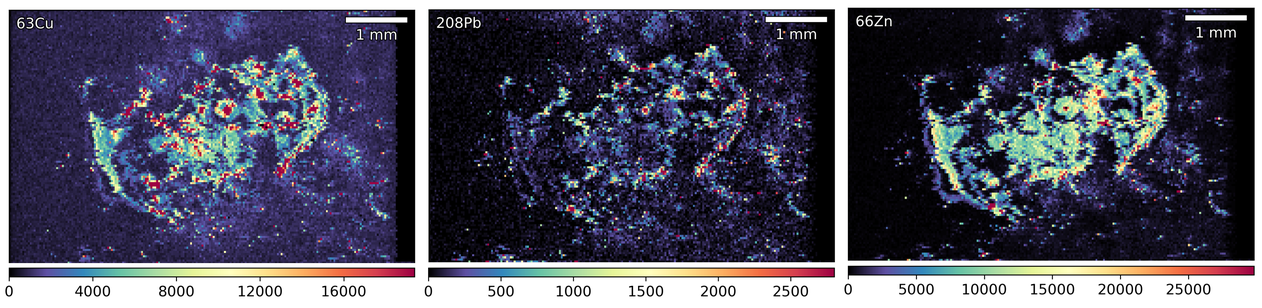

Figure 2. Preliminary analysis results: 2-dimensional mapping of Cu (left), Pb (middle) and Zn (right) in a 30 µm slice of an adult forager bee brain (not dosed).

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References
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