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Spatial and temporal variation of metal concentrations in adult honeybees (*Apis mellifera* L.)

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Abstract Honeybees (*Apis mellifera* L.) have great potential for detecting and monitoring environmental pollution, given their wide-ranging foraging behaviour. Previous studies have demonstrated that concentrations of metals in adult honeybees were significantly higher at polluted than at control locations. These studies focused at a limited range of heavy metals and highly contrasting locations, and sampling was rarely repeated over a prolonged period. In our study, the potential of honeybees to detect and monitor metal pollution was further explored by measuring the concentration in adult honeybees of a wide range of trace metals, nine of which were not studied before, at three locations in the Netherlands over a 3-month period. The specific objective of the study was to assess the spatial and temporal variation in concentration in adult honeybees of Al, As, Cd, Co, Cr, Cu, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, V and Zn. In the period of July–September 2006, replicated samples were taken at 2-week intervals from commercial-type beehives. The metal concentration in micrograms per gram honeybee was determined by inductive coupled plasma–atomic emission spectrometry. Significant differences in concentration between sampling dates per location were found for Al, Cd, Co, Cr, Cu, Mn Sr, Ti and V, and significant differences in average concentration between locations were found for Co, Sr and V. The results indicate that honeybees can serve to detect temporal and spatial patterns in environmental metal concentrations, even at relatively low levels of pollution.

Keywords Metals · Bioindication · Pollution · Honeybee

Introduction

Bioindication is a time-dependent, sensitive registration of anthropogenic or anthropogenically altered environmental factors, by distinguished dimensions of biological objects and biological systems under defined circumstances (Stöcker 1980). Honeybees (*Apis mellifera* L.) are potentially highly useful as bioindicators in detecting and monitoring environmental pollution, given their worldwide usage for honey production and pollination and their wide-ranging foraging behaviour (Bromenshenk and Preston 1986; Raeymaekers 2006). Not surprisingly, studies on the use of honeybees and bee products for environ-

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mental monitoring have a relatively long history, dating back to at least 1935 (Crane 1984). Environmental pollutants included in these studies were, among others, pesticides, radioactive elements and heavy metals (Devillers and Pham-Delègue 2002). As for the latter, honeybees may take up heavy metals from all environmental compartments: soil, vegetation, air and water (Bromenshenk et al. 1985; Porrini et al. 2003). Heavy metals end up in these compartments after emission from a variety of mainly anthropogenic sources. A major source of heavy metals in the atmosphere, for example, is the combustion of fossil fuels which results in the emission of ultrafine metal-containing particles. These airborne particles eventually deposit on vegetation, soil or surface water. Honeybees pick up heavy metals from the environment through a wide range of pathways: by ingestion of polluted surface water, pollen and nectar, by impaction and inhalation of particles during flight and by adhesion of particles to their hairy bodies when moving over plant and soil surfaces during foraging. In this way, honeybees provide an integrated sample of the environmental compartments in the area within their flight range (c. 7 km^2, Bromenshenk et al. 1985), and can therefore serve to indicate anomalies in the environmental distribution of trace metals in time and space (Raeymaekers 2006). Possible mechanisms behind detected anomalies can then be studied with other, more specific methods.

Heavy metals in bees and in bee products have been the subject of many studies (e.g. Bromenshenk et al. 1985; Conti and Botré 2001; Fakhimzadeh and Lodenius 2000; Kalnins and Detroy 1984; Leita et al. 1996; Roman 2005; Veleminsky et al. 1990). The most frequently studied metals were lead, cadmium, chromium, copper and zinc, which are known pollutants from transport and industrial activity, disseminated via combustion gases. Most studies focused on a limited number of metals and highly contrasting locations, and sampling was rarely repeated over a prolonged period. In our study, the potential of honeybees to detect and monitor metal pollution was further explored by measuring the concentration in adult honeybees of a wide range of 18 trace metals, 9 of which were not studied before, at three locations over a 3-month period. The specific objective of the study was to assess the spatial and temporal variation in concentration in adult honeybees of aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lithium (Li), manganese (Mn), molybdene (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), titanium (Ti), vanadium (V) and zinc (Zn). We chose to study the metal concentrations in adult worker bees as these are considered to provide more sensitive, reliable and up-to-date information about exposure of bees to metals in the environment than concentrations in pollen and honey (Bromenshenk et al. 1985; Jones 1987; Fakhimzadeh and Lodenius 2000; Porrini et al. 2002; Veleminsky et al. 1990).

Materials and methods

At three locations in the Netherlands, three honeybee colonies (replicates) per location were placed. During a 3-month period, from July to September 2006, samples of each honeybee colony were taken every 14 days. The concentration of metals in honeybees was determined chemically.

Study locations

Location Maastricht, Limburglaan

The study was conducted in Maastricht, Limburglaan. Maastricht is an urban area with cement industry and glass industry, and is located close to large industrial areas such as Liège in Belgium. The honeybee colonies were placed near the city centre, on the roof of the provincial government building.

Location Buggenum, Dorpstraat

Buggenum is a village in a rural area about 60 km north of Maastricht. In Buggenum, bricks are produced, and a large electric power plant is situated next to the village. This plant is powered by coal, natural gas and biomass.

Location Hoek van Holland, Prins Hendrikstraat

Hoek van Holland is situated in the Rijnmond region at the river mouth of the Nieuwe Waterweg, at the North Sea coast. The Rijnmond region includes the port of Rotterdam and a large industrial area where, among others, petrochemical industry, tank storage and tank transfer and waste treatment plants are situated.

Honey bee sampling method

Honeybee colonies were kept from winter till summer in the same apiary in Wageningen (The Netherlands) till distribution over the three locations. At each location, three honeybees colonies (*A. mellifera*) were placed. The colonies were kept in one-storey wooden hives with ten frames (Simplex measures NEN 061–50). This is the most commonly, commercially used type of hive in the Netherlands. During the study period of July, August and September 2006, every 2 weeks, a random sample of 100 to 150 worker honeybees was taken from the outer frame of the hive that was occupied with bees but without brood. Sampling was done by brushing bees with a plastic brush into a plastic container. This resulted in 18 bee samples per location (three replicates of six sampling dates) to be analysed for all metals per location. The samples were transported in a cooler box and stored in the freezer at −20±5°C until analysis.

Measurement of heavy metal concentrations in bees

The chemical analyses on metals were carried out by the environmental research laboratory of the Province of Limburg (Hoofdgroep Milieu en Water, Bureau onderzoek en advies), using the inductive coupled plasma–atomic emission spectrometry (ICP-AES) technique. From each sample (i.e. from each combination of colony, sampling date and location), 25 frozen worker bees were taken at random from the sample. The bees were subsequently weighed, dried for 24 h at 120°C, weighed and destructed by boiling the sample at 170°C in a mixture of 25 ml HNO_3 (70%) and HCl (37%) at a ratio of 1:3 (aqua regia). The resulting liquid was topped up to 50 ml with demi water. Five milliliters of the 50 ml was filtered over a cotton wool filter and analysed using ICP-AES. The resulting signals (nanograms per millilitre) were converted to nanograms per gram (parts per billion (ppb)) bee with a conversion factor (volume sample/(weight bees × mean percentage dry weight)) resulting in ppb metal dry weight which was subsequently converted to micrograms per gram bee (micrograms per gram dry material (dm)). The overall weight loss of the bee samples as a result of the drying process was 68%.

Statistical analyses

Per metal, a generalized linear mixed model analysis was done assuming a lognormal distribution of the concentration data. Differences in concentrations between dates or locations were considered significant at P values ≤ 0.05, using Tukey's multiple comparisons test. Temporal differences in concentration were assessed by comparing the 2-week sample values for each location. Spatial differences in concentration were assessed by comparing the mean values of the entire 3-month study period between locations.

Results and discussion

Temporal and spatial variation in metal concentrations

The 2-week sample values of metal concentrations (averages of the three replicate samples) are presented for each location in Table 1. Different lowercase letters indicate per location (row) statistically significant differences between metal concentrations in samples taken at different dates. For nine of the metals included in our study, no differences between 2-week sample values were found, and the concentrations were apparently constant over time. For the other nine metals (Al, Cd, Co, Cr, Cu, Mn, Sr, Ti and V), significant differences between 2-week sample values were found in at least one of the study locations. The fluctuations in concentration indicate a significant variation in exposure of honeybees to these metals in the environment.

For all but three metals, no significant differences in mean concentration (over the entire study period) between locations could be detected. This indicates that the overall environmental exposure of honeybees to the metals Al, As, Cd, Cr, Cu, Li, Mn, Ni, Pb, Sb, Se, Sn, Ti and Zn during the study period was comparable in Maastricht, Buggenum and Hoek van Holland. The overall mean concentrations of Co, Sr and V, however, differed significantly between the study locations (Table 2). These spatial differences might be caused by differences in industrial activity near these locations. Markedly more significant temporal (nine) than spatial (three) differences were found. This probably indicates that the temporal fluctuations in source strength over a 3-month period are greater than the more structural differences between locations. In a

Table 1 Metal concentrations in worker honeybees (micrograms per gram dry matter) from three locations, sampled at 2-week intervals (July–September 2006)

Element	Location	Sampling date					
		20 July	3 Aug	17 Aug	31 Aug	14 Sept	28 Sept
Al	Maastricht	15.10b	10.75ab	5.9a	6.8a	9.89ab	9.3ab
Al	Buggenum	11.55bc	10.93bc	4.6a	6.6ab	15.52c	11.07bc
Al	Hoek van Holland	10.70bc	13.20c	6.15ab	5.57a	12.17c	9.49abc
As	Maastricht	0.72a	0.70a	0.66a	0.76a	0.68a	0.83a
As	Buggenum	0.70a	0.69a	0.77a	0.76a	0.70a	0.71a
As	Hoek van Holland	0.68a	0.67a	0.69a	0.69a	0.69a	0.75a
Cd	Maastricht	0.09ab	0.09ab	0.07a	0.17ab	0.24ab	0.75b
Cd	Buggenum	0.14a	0.25a	0.10a	0.18a	0.19a	0.71a
Cd	Hoek van Holland	0.13ab	0.06ab	0.05a	0.05a	0.50b	0.25ab
Co	Maastricht	0.10a	0.10a	0.08a	0.11a	0.14a	0.12a
Co	Buggenum	0.26ab	0.21ab	0.16a	0.33b	0.16a	0.16a
Co	Hoek van Holland	0.10a	0.11a	0.09a	0.10a	0.11a	0.09a
Cr	Maastricht	0.27b	0.21ab	0.16a	0.18ab	0.24ab	0.23ab
Cr	Buggenum	0.23ab	0.23ab	0.15a	0.21ab	0.25ab	0.28b
Cr	Hoek van Holland	0.27ab	0.22ab	0.18a	0.18a	0.28b	0.22ab
Cu	Maastricht	14.69a	18.37a	19.16a	16.86a	17.64a	19.74a
Cu	Buggenum	12.69ab	11.65a	11.85a	15.50ab	12.57ab	19.77b
Cu	Hoek van Holland	14.21a	14.33a	12.84a	13.13a	15.23a	15.80a
Li	Maastricht	0.05a	0.02a	0.01a	0.01a	0.02a	0.02a
Li	Buggenum	0.03a	0.02a	0.02a	0.01a	0.02a	0.03a
Li	Hoek van Holland	0.05a	0.04a	0.01a	0.01a	0.03a	0.01a
Mn	Maastricht	24.45ab	28.31ab	20.69a	41.98ab	68.76b	45.10ab
Mn	Buggenum	31.04a	28.42a	29.16a	47.34a	48.40a	50.80a
Mn	Hoek van Holland	32.11a	30.44a	26.48a	28.87a	34.48a	34.37a
Mo	Maastricht	0.77a	1.16a	1.07a	0.64a	0.73a	0.54a
Mo	Buggenum	0.53a	0.42a	0.36a	0.57a	0.75a	0.66a
Mo	Hoek van Holland	0.55a	0.55a	0.51a	0.50a	0.68a	0.46a
Ni	Maastricht	0.37a	0.44a	0.34a	0.26a	0.22a	0.19a
Ni	Buggenum	0.29a	0.47a	0.25a	0.28a	0.29a	0.29a
Ni	Hoek van Holland	0.43a	0.35a	0.29a	0.26a	0.41a	0.20a
Pb	Maastricht	0.41a	0.37a	0.26a	0.31a	0.55a	1.26a
Pb	Buggenum	0.27a	1.10a	0.19a	0.30a	0.53a	0.58a
Pb	Hoek van Holland	1.00a	0.30a	0.27a	0.35a	1.67a	0.55a
Sb	Maastricht	0.12a	0.10a	0.11a	0.11a	0.18a	0.13a
Sb	Buggenum	0.11a	0.10a	0.12a	0.15a	0.09a	0.12a
Sb	Hoek van Holland	0.19a	0.07a	0.11a	0.09a	0.10a	0.11a
Se	Maastricht	1.38a	1.23a	1.24a	1.30a	1.50a	1.53a
Se	Buggenum	1.35a	1.27a	1.24a	1.38a	1.28a	1.22a
Se	Hoek van Holland	1.24a	1.20a	1.21a	1.17a	1.15a	1.17a
Sn	Maastricht	0.51a	0.44a	0.47a	0.44a	0.62a	0.52a
Sn	Buggenum	0.54a	0.68a	0.49a	0.43a	0.50a	0.42a
Sn	Hoek van Holland	0.76a	0.47a	0.51a	0.47a	0.44a	0.44a

Table 1 (continued)

Element	Location	Sampling date					
		20 July	3 Aug	17 Aug	31 Aug	14 Sept	28 Sept
Sr	Maastricht	1.82ab	2.99b	1.54ab	0.95a	1.05a	1.00a
Sr	Buggenum	0.99a	1.02a	0.70a	0.86a	0.89a	1.00a
Sr	Hoek van Holland	2.18a	2.40a	1.97a	1.36a	1.33a	0.94a
Ti	Maastricht	0.45b	0.37ab	0.16a	0.22ab	0.43b	0.47b
Ti	Buggenum	0.34bc	0.41bc	0.09a	0.17ab	0.55c	0.39bc
Ti	Hoek van Holland	0.54c	0.51bc	0.20ab	0.17a	0.50bc	0.35abc
V	Maastricht	0.040ab	0.032ab	0.015a	0.015a	0.054b	0.033ab
V	Buggenum	0.028b	0.026b	0.006a	0.006a	0.042b	0.029b
V	Hoek van Holland	0.083a	0.14b	0.10a	0.093a	0.31b	0.31b
Zn	Maastricht	67.81a	72.36a	59.18a	72.03a	82.83a	100.46a
Zn	Buggenum	73.66a	75.54a	70.70a	94.52a	71.60a	95.44a
Zn	Hoek van Holland	63.38a	68.98a	61.61a	61.14a	71.49a	74.76a

Concentration values are calculated as means of three independent replicate samples

Different lowercase letters indicate per location (row) statistically significant differences between metal concentrations in samples taken at different days

Table 2 Metal concentrations in worker honeybees (micrograms per gram dry matter) in samples from three locations

Element	Maastricht	Buggenum	Hoek van Holland
Al	9.17a	9.33a	9.07a
As	0.72a	0.73a	0.69a
Cd	0.16a	0.21a	0.11a
Co	0.11a	0.21b	0.10a
Cr	0.21a	0.22a	0.22a
Cu	17.66a	13.75a	14.22a
Li	0.02a	0.02a	0.02a
Mn	35.08a	37.97a	30.99a
Mo	0.79a	0.53a	0.54a
Ni	0.30a	0.31a	0.31a
Pb	0.45a	0.42a	0.55a
Sb	0.12a	0.11a	0.11a
Se	1.36a	1.29a	1.19a
Sn	0.50a	0.51a	0.51a
Sr	1.42ab	0.90a	1.61b
Ti	0.33a	0.28a	0.34a
V	0.03a	0.02a	0.15b
Zn	74.72a	79.59a	66.70a

Concentration values are calculated as sample means over the entire study period (7 July to September 2006)

Different lowercase letters indicate per location (row) statistically significant differences between sample means over the entire study period

small and densely populated country as the Netherlands, spatial differences may be expected to be limited. However, as our method does not provide information on sources and mechanisms, any explanation of the observed differences, spatial as well as temporal, will remain speculative. In case the variations in metal concentrations in time and space detected with honeybees are considered to be a reason of concern, other, more specific methods will have to be used to investigate the causal mechanisms. For example, use could be made of the Enrichment Factor (Chester et al. 1999), to determine whether trace metals in the air have significant non-crustal sources.

Comparison with previously reported concentrations

For Al, Co, Li, Mo, Sb, Sn, Sr, Ti and V, no previous reports on their concentrations in adult honeybees have been published. The ranges of the concentrations of these metals as found in our study are as follows: Al, 4.6–15.52 μg g^{-1}; Co, 0.08–0.33 μg g^{-1}; Li, 0.01–0.05 μg g^{-1}; Mo, 0.36–1.16 μg g^{-1}; Sb, 0.07–0.19 μg g^{-1}; Sn, 0.44–0.76 μg g^{-1}; Sr, 0.70–2.18 μg g^{-1}; Ti, 0.09–0.55 μg g^{-1}; and V, 0.006–0.31 μg g^{-1}. For As, Cd, Cr, Cu, Mn, Ni, Pb, Se and Zn, published reports on concentrations in adult honeybees are available from a wide variety of

Table 3 Metal concentrations in adult honeybees in the current and previous studies

Element	Current study	Previous studies		
	Concentration range (μg g^{-1})	Concentration range (μg g^{-1})	Comments	Reference
As	0.67–0.83	<0.5–12.5	72 sites (rural–urban)	Bromenshenk et al. 1985
		<0.1	Hives without CCA	Kalnins and Detroy (1984)
		0.77–1.11	Hives with CCA	Kalnins and Detroy (1984)
Cd	0.05–0.75	<0.6–>1.8	72 sites (rural–urban)	Bromenshenk et al. 1985
		2.89–3.43	Non-contaminated sites	Conti and Botré 2001
		2.87–4.23	Sites in city centre/near highway	Conti and Botré 2001
		0.03–0.18	Control sites	Fakhimzadeh and Lodenius (2000)
		0.05–1.2	Industrial sites	Fakhimzadeh and Lodenius (2000)
		1.1–1.9[a]	Near crossroad with heavy traffic	Leita et al. (1996)
		0.14–0.16[a]	Agricultural-forest region	Roman (2005)
		0.10–0.17[a]	Industrialized region	Roman (2005)
		0.16–1.34	Relatively clean locality	Veleminsky et al. (1990)
		0.74–1.75	Industrial locality	Veleminsky et al. (1990)
Cr	0.15–0.28	0.054–0.080	Non-contaminated sites	Conti and Botré 2001
		0.052–0.116	Sites in city centre/near highway	Conti and Botré 2001
		1.4±0.2[a]	Different locations	Kump et al. (1996)
		<0.06–0.34	Hives without CCA	Kalnins and Detroy (1984)
		0.58–0.8	Hives with CCA	Kalnins and Detroy (1984)
		<0.1–3.6	National park	Porrini et al. (2002)
		<0.1–1.2	City centre	Porrini et al. (2002)
		0.05–0.18[a]	Agricultural-forest region	Roman (2005)
		0.16–0.23[a]	Industrialized region	Roman (2005)
Cu	11.65–19.77	13–15	Control sites	Fakhimzadeh and Lodenius (2000)
		14–27	Industrial sites	Fakhimzadeh and Lodenius (2000)
		35.7±1.5[a]	Different locations	Kump et al. (1996)
		8.68–9.70	Hives without CCA	Kalnins and Detroy (1984)
		9.86–10.5	Hives with CCA	Kalnins and Detroy (1984)
		15.16–30.55	Relatively clean locality	Veleminsky et al. (1990)
		31.89–37.68	Industrial locality	Veleminsky et al. (1990)
Mn	20.69–50.80	75.7±5.6[a]	Different locations	Kump et al. (1996)
Ni	0.19–0.47	0.12–0.42	National park	Porrini et al. (2002)
		0.13–0.43	City centre	Porrini et al. (2002)
		0.27–0.42[a]	Agricultural-forest region	Roman (2005)
		0.36–0.50[a]	Industrialized region	Roman (2005)
Pb	0.19–1.67	0.52–1.00	Non-contaminated sites	Conti and Botré 2001
		0.64–1.25	Sites in city centre/near highway	Conti and Botré 2001
		0.58–0.62	Control sites	Fakhimzadeh and Lodenius (2000)
		0.27–1.4	Industrial sites	Fakhimzadeh and Lodenius (2000)
		1.4–3.0[a]	Near crossroad with heavy traffic	Leita et al. (1996)
		0.15–0.55	National park	Porrini et al. (2002)

Table 3 (continued)

Element	Current study	Previous studies		
	Concentration range (μg g^{-1})	Concentration range (μg g^{-1})	Comments	Reference
		0.45–0.95	City centre	Porrini et al. (2002)
		1.5–30	Far from–near busy highway	Pratt and Sikorski (1982)
		0.28–0.29[a]	Agricultural-forest region	Roman (2005)
		0.64–1.01[a]	Industrialized region	Roman (2005)
		0.58–2.47	Relatively clean locality	Veleminsky et al. (1990)
		3.68–9.28	Industrial locality	Veleminsky et al. (1990)
Se	1.15–1.53	1.84–2.38[a]	Agricultural-forest region	Roman (2005)
		2.16–5.98[a]	Industrialized region	Roman (2005)
Zn	61.14–100.64	55–73	Control sites	Fakhimzadeh and Lodenius (2000)
		59–100	Industrial sites	Fakhimzadeh and Lodenius (2000)
		202±5[a]	Different locations	Kump et al. (1996)
		52.5–76.2[a]	Near crossroad with heavy traffic	Leita et al. (1996)
		90.34–188.72	Relatively clean locality	Veleminsky et al. (1990)
		153.34–204.4	Industrial locality	Veleminsky et al. (1990)

[a] ICP-AES analyses

sampling locations. These values are presented in Table 3, together with the ranges of concentrations found in our study. The method of analysis to determine metal concentrations in bees was either ICP-AES, as in our study, or atomic absorption spectrometry. Kump et al. (1996) compared both methods for Cr, Cu, Mn, Ni, Pb and Zn and found only significant differences between these methods for Cr. The concentrations of the metals measured in our study are all within the bandwidth of the values reported in the literature, with the exception of Mn and Se. The concentrations we found for these two metals are lower than reported from other studies, but in the same order of magnitude. In general, the metal concentrations in the present study were at the lower or middle-lower end of the spectrum of concentration values found in other studies and often match the range of concentrations reported for supposedly relatively 'clean' locations. This indicates that the level of metal pollution at our three study locations was relatively low. The high concentrations, in absolute terms, of Cu, Mn and Zn are comparable to values found in other studies and are most likely due to the relatively high natural concentrations of these metals in pollen (Lambers et al. 1998), on which the bees feed.

Sources of metals in the hive environment

The commercial-type beehives used in our experiment have metal or metal-based components, such as stainless steel frame holders and wood-preserving coatings. ICP-AES analysis of samples from the frame holders and from paint of the landing board at the hive entrance revealed traces of As, Cr, Cu and Ni in both types of material (L. Goessen, personal communication). Thus, we cannot exclude that at least part of the load of these metals in the sampled bees originated from hive-associated sources. Kalnins and Detroy (1984) studied the effect of the use of the wood preservative chromated copper arsenate (CCA) in hives on the concentrations of As, Cr and Cu in bees. They found that the use of CCA significantly enhanced the concentrations of As and Cr in bees, but the concentrations of Cu were not significantly affected (Table 3). The lack of effect on Cu is probably due to the much greater importance of pollen as a source of this metal in bees. The concentrations of As found in our study correspond with those from hives treated with CCA in Kalnins and Detroy's study (1984) and are therefore probably the consequence of exposure to hive-associated

sources rather than to sources in the external environment. As for Cr, the concentrations found in our study correspond with those from hives not treated with CCA, suggesting that the Cr-containing materials of the hive were not an important source of contamination in this case.

Conclusion

Our results indicate that honeybees can serve to detect temporal and spatial patterns in environmental metal concentrations, even at relatively low levels of pollution. A restriction on the potential use of apiculture for biomonitoring of metal pollution is posed by the application of metal components and metal-based wood preservatives in commercial-type beehives.

A next step will be to determine sources of variation in metal concentrations. For that purpose, larger scale studies are required that allow for detailed statistical analysis. For example, we will conduct a follow-up study on spatial variation of metal concentrations in honeybees covering 150 locations across the Netherlands, which will be analysed with geostatistical methods.

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