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Honey, Pollen and Bees as Indicator of Metal Pollution

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Abstract: The objective of this study was to determine the degree of heavy metal (Cd, Pb, Cu, Fe, Mn, and Zn) pollution in Finnish honey, pollen and bees, and to evaluate their use as biological indicators of environmental pollution. Twelve sites representing the three categories: industrial, urban and control areas around Finland were sampled (four in each category). The metal concentrations in honey were very low at all study sites. No significant differences were found in metal pollution in honey or in pollen between these categories. The level of Cd, Cu, Zn and Fe in bees were significantly different between these categories. It was concluded that under Finnish conditions honey and pollen are not good bio-indicators of heavy metal pollution, but bees can be used for biomonitoring purposes at least for these metals. The average level of Cd, Cu, Zn and Fe that bees posses in industrial sites were 0.423 :g g^{-1}, 19 :g g^{-1}, 76.25 :g g^{-1} and 172.5 :g g^{-1} respectively.

Keywords: metal, bee, honey, pollen, bioindicator.

INTRODUCTION

Metals are emitted from a great number of sources which contribute to metal loads in terrestrial and aquatic food chains. The spread of heavy metals on vegetables and fruits may directly end up into our food chain. Another incidental way of metals getting into our food chain is through their spread over flowering plants. Metal polluted pollen may elevate metal concentrations in honey.

Many insects have been used as biomonitors of air pollution by heavy metals (e.g. Heliövaara *et al.* 1990, Heliövaara and Väisänen 1993). Hive products have also been considered useful for assessing environmental pollution (Leita *et al.* 1996, Jablonski *et al.* 1995). Psyllids accumulate low amounts of metals in general but their metal burdens increase with age. Jumping plant lice (psyllids) produce considerable amounts of polluted honeydew that may be gathered by honey bees. In polluted areas *Psyllopsis* *fraxini* excrete large amounts of Al, Fe, Cu, Mn, and Cd with honeydew (Glowacka *et al.* 1997). Crawford *et al.* (1995) demonstrated the accumulation of Cd in aphids while Cu concentrations were significantly lower in aphids than in the honeydew.

Dobrzanski *et al.* (1994) studied Polish honey and found the following concentrations: Pb 0.09-1.9 :g g^{-1}, Cd 0.01-0.11 :g g^{-1}, Zn 0.34-7.2 :g g^{-1} and Cu 0.13-0.35 :g g^{-1}. Polish pollen always contained 30 – 100% more lead, cadmium and copper than honey did (Jablonski *et al.* 1995, Migula *et al.* 1989). In Italy Cesco *et al.* (1994) found dead bees to be more polluted by Pb and Cd than pollen. Large amounts of Zn and Cd, but not Pb, have been found on the surface of bees' bodies (Leita *et al.* 1996). Veleminsky *et al.* (1990) considers foragers returning to the hive to be the most useful tool for environmental monitoring.

The objectives of this study were to determine the degree and extent of heavy metal pollution in Finnish honey, pollen and bees, and to evaluate the suitability of these items as bioindicators of airborne metal pollution.

MATERIAL AND METHODS

Study areas and sampling

Honey, pollen and adult honey bees (*Apis mellifera*) were sampled from twelve sites throughout Finland (Figure 1). The levels of metal pollution at the sampling sites were estimated on basis of metal concentrations in moss (Rühling 1994) and knowledge of main pollution sources (Kubin et al. 1996, Melanen et al. 1999). We classified the pollution level into three categories of industrial, urban and control with four sites in each category. Äetsä, Harjavalta, Kokkola, and Imatra were the industrial sites. Vaasa,

Table 1. Sampling sites, number of observations and possible sources of metal pollution. Industrial, *urban and control sites are indicated with, (I), (U) and asterisks (*) respectively*

Sampling site	n	Possible pollution sources
a) Äetsä (I)	6	Chlor alkali plant
b) Harjavalta (I)	6	Copper nickel smelter
c) Vaasa (U)	6	Foundry industries
d) Kokkola (I)	6	Zinc industries
e) Raahe (U)	4	Steel industries
f) Kemi*	4	Control site
g) Oulu (U)	6	Paper industry, traffic
h) Kuhmo*	6	Control site
i) Joensuu*	4	Control site
j) Imatra (I)	6	Steel industry
k) Kouvola*	6	Control site
l) Salo (U)	2	Traffic

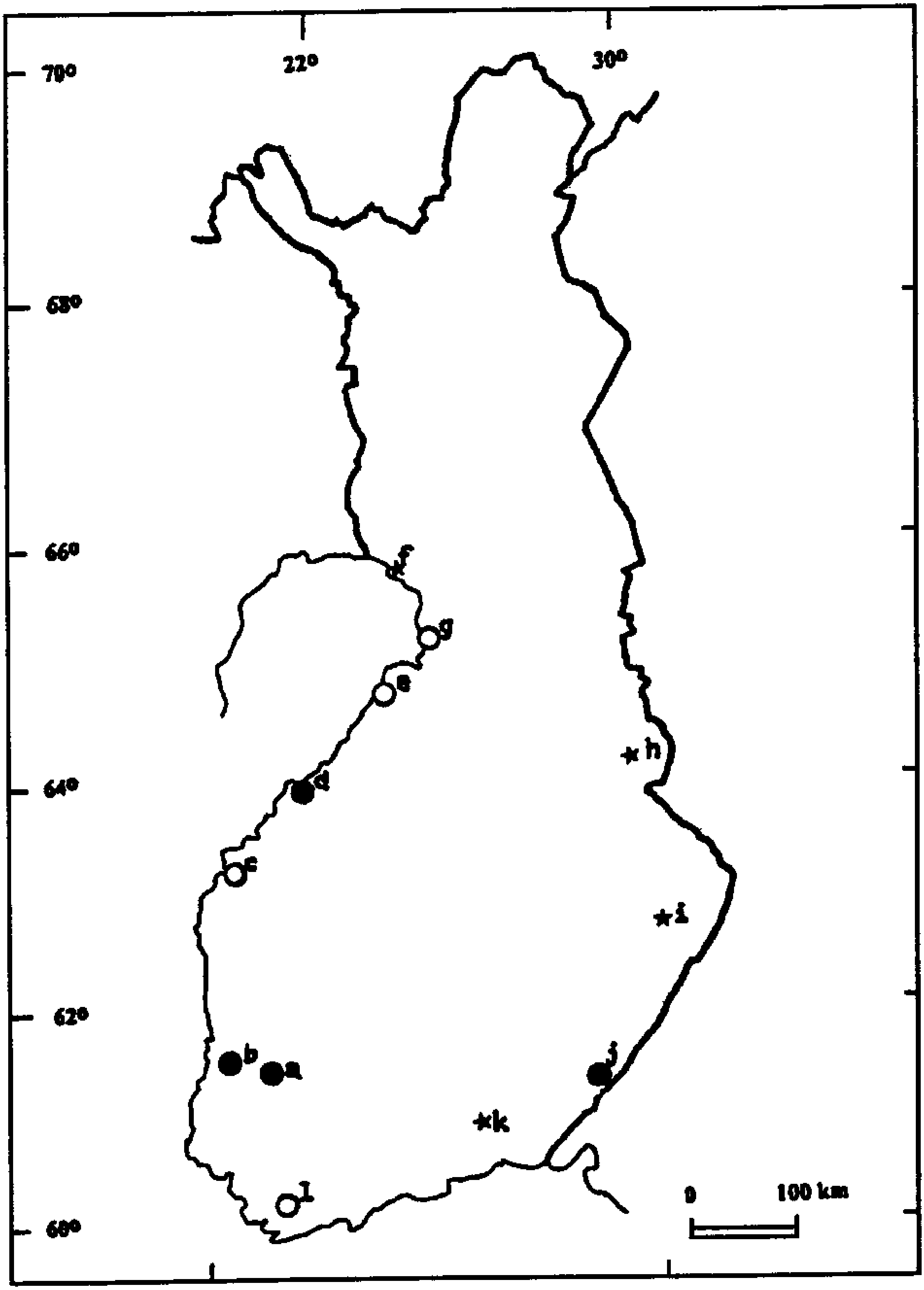

Fig. 1. Areas studied in Finland. Industrial, urban and control sites are marked by filled circles, empty circles and stars respectively. The letters "a" – "l" refers to the sampling sites explained in table 1.

Raahe, Oulu and Salo were in the urban category and the rest of the sites were in the control category (Kemi, Kuhmo, Joensuu and Kouvola). Since the main honey flow in Finland is usually in July, colonies of honey bees (*Apis mellifera*) were sampled after mid July 1994.

To avoid contamination plastic gloves were used in sampling and handling of samples. The honey was collected by squeezing a comb honey into a small (60 ml) disposable plastic container. Pollen samples were collected by cutting a piece of comb (6 cm^2) containing stored pollen (in both sides of the comb) by a disposable plastic knife. Bees were brushed directly into a disposable plastic bag (30 bees) from the honey combs located on the farthest side walls of the hive. These are normally old bees probably more exposed to airborne pollution. All samples were kept at room temperature during the 6 days' sampling trip and transferred to a freezer -20 °C after the trip.

Analytical methods

Honey samples were homogenized and oven-dried over night at 105 °C on petri dishes. Next morning while the honey was still hot, it was pulled up with the help of a glass rod and cooled with cold air. Honey dried within a few seconds through this method and pure honey was obtained. In order to obtain pollen from a comb, pollen samples were kept in a freezer at -20 °C over night. The pollen was removed quickly by breaking the comb while the wax was brittle. The pollen obtained were then dried overnight at 105 °C. The bee samples were first placed in freezer after the collection trip. They were then dried overnight at 105 °C. All samples were further dried in a desiccator for 24 h.

Pollen and honey samples to be analysed by flame technique or graphite furnace AAS technique were digested according to the following procedure: 0.5 g sub-samples were weighed and digested in 5 ml 65 % pure nitric acid HNO_3 (BDH, Aristar) for 1 h at 75 °C, 4 h at 105 °C and 4 h at 170 °C. The digested samples were filtered and diluted to 25 ml with distilled water (Nuorteva 1990). The method has been tested using standard reference material (Table 2).

Table 2. Certified and obtained values (:g g-1 dry wt) for the BCR standard reference material „Cod muscle" CRM-422. (C.I.= Confidence Interval, S.D.= Standard Deviation)

	Certified value mean ± 95 % C.I.	Obtained value mean ± S.D.; N = 6	Our detection limit
Cd	0.017 ± 0.002	0.018 ± 0.003	0,005
Cu	1.05 ± 0.07	2.0 ± 1.0	1
Fe	5.46 ± 0.30	6.7 ± 1.8	2
Mn	0.543 ± 0.028	0.59 ± 0.15	0,4
Pb	0.085 ± 0.015	< 0.2	0,2
Zn	19.6 ± 0.5	20.9 ± 1.5	1

The dried bee samples (no milling) were removed from the desiccator and weighed to -0.5 g and dissolved into 5 ml of HNO_3 (BDH, Aristar) for 2 h at 50°C, and after that for 16-18 h at 110 °C. Five ml of H_2O_2 were added, and the samples were heated for an additional 6 h. The samples were filtered and diluted with distilled water to 25 ml. Finally, the sample solutions were analysed for their metal concentrations by a flame atomic absorption spectrophotometer (Varian SpectrAA-400) or by graphite furnace AAS (Varian SpectrAA400 equipped with GTA-96).

Statistical procedures

Analysis of variance procedures were applied using one-way ANOVA. Paired t-test was used for further analysis of the data. The pairs were samples of polluted areas vs control areas, unless explained otherwise. The t-test was referred to by lowercase t. 5, 1, and 0.1% risk levels are indicated by *, **, ***, respectively. Pearson correlation was used between the metal concentrations in bees and estimated pollution levels.

RESULTS AND DISCUSSION

At all sites the concentrations of heavy metals in honey were very low (Table 3). Most concentrations of cadmium, copper and lead were under our detection limits. Our results were at the same level or lower than concentrations found in Polish honey (Bogdanov *et al.* 1986). The pollen samples (Table 4) contained higher concentrations than the honey samples. This is in agreement with Polish (Migula *et al.* 1989) and German (Müller and Agthe 1988) studies where higher concentrations were found in pollen than in honey samples. The concentrations of heavy metals in pollen were still generally under that accepted level for food material in Finland. In a few cases the mean value was above the accepted levels: Cu in Harjavalta (site b) and Kuhmo (h), Pb in Kokkola (d) and Cd in Raahe (e). However, the ANOVA of mean values of heavy metals in pollen in each site showed no statistical differences between the sites.

Table 3. Metal concentrations (:g g^{-1} of dry weight; arithmetic means ± SD) in honey at our sampling sites "a" – "l" (see Figure 1). Industrial, urban and control sites are indicated with, (I), (U) and asterisks (*) respectively

Site	Fe	Mn	Cu	Zn	Cd	Pb
a (I)	2.1 ± 2.8	0.58 ± 0.38	< 1.0	2.1 ± 1.1	< 0.005	< 0.2
b (I)	< 2.0	1.6 ± 1.1	< 1.0	2.4 ± 0.45	0.01 ± 0.01	< 0.2
d (I)	< 2.0	1.4 ± 0.24	< 1.0	3.9 ± 4.4	< 0.005	< 0.2
j (I)	< 2.0	1.2 ± 0.87	< 1.0	1.9 ± 1.6	< 0.005	< 0.2
c (U)	< 2.0	3.2 ± 1.0	< 1.0	1.6 ± 0.43	< 0.005	< 0.2
e (U)	< 2.0	0.67 ± 0.08	< 1.0	1.8 ± 0.40	< 0.005	< 0.2
g (U)	3.4 ± 6.8	1.34 ± 0.47	< 1.0	< 1.0	< 0.005	< 0.2
i (U)	2.4 ± 0.30	< 0.4	< 1.0	< 1.0	< 0.005	< 0.2
f *	< 2.0	0.73 ± 0.38	< 1.0	1.2 ± 0.74	< 0.005	< 0.2
h *	< 2.0	3.1 ± 1.5	< 1.0	< 1.0	< 0.005	0.2 ± 0.7
l *	< 2.0	2.3 ± 0.23	< 1.0	< 1.0	< 0.005	< 0.2
k *	< 2.0	1.5 ± 0.89	< 1.0	1.1 ± 0.50	< 0.005	< 0.2

Table 4. Metal concentrations (:g g-1 of dry weight; arithmetic means ± SD) in pollen at our sampling sites "a" – "l" (see Figure 1). .Industrial, urban and control sites are indicated with, (I), (U) and asterisks (*) respectively

Site	Fe	Mn	Cu	Zn	Cd	Pb
a (I)	48 ± 20	56 ± 12	9.0 ± 0.76	35 ± 4.4	0.04 ± 0.02	< 0.2
b (I)	76 ± 8.7	45 ± 19	19 ± 6.1	35 ± 3.8	0.06 ± 0.05	< 0.2
d (I)	120 ± 34 92	± 45	9.4 ± 1.2	49 ± 8.2	0.09 ± 0.04	0.37 ± 0.25
j (I)	47 ± 30	27 ± 5	7.8 ± 1.1	37 ± 5.6	0.07 ± 0.07	0.28 ± 0.09
c (U)	44 ± 7.4	60 ± 33	7.9 ± 2.0	29 ± 3.7	0.03 ± 0.01	< 0.2
e (U)	110 ± 140	73 ± 20	8.4 ± 1.1	49 ± 4.3	0.15 ± 0.03	0.24 ± 0.10
g (U)	110 ± 36	73 ± 7	9.9 ± 0.88	43 ± 3.2	0.06 ± 0.05	0.24 ± 0.18
l (U)	60 ± 5	21 ± 3	9.2 ± 0.48	38 ± 0.32	0.01 ± 0.00	< 0.2
f *	69 ± 15	68 ± 18	6.8 ± 0.93	35 ± 6.0	0.04 ± 0.02	< 0.2
h *	59 ± 11	110 ± 55	12 ± 1.1	49 ± 5.9	0.02 ± 0.01	< 0.2
i *	50 ± 28	70 ± 29	8.9 ± 0.52	34 ± 1.4	0.03 ± 0.01	0.20 ± 0.02
k *	48 ± 28	86 ± 81	10 ± 1.9	46 ± 8.5	0.04 ± 0.03	< 0.2

The levels of heavy metals were higher in bees than in pollen and honey (Table 5). The results of the ANOVA showed that the differences between the experimental categories in Cd pollution in bees are statistically highly significant (F = 7.87 P = 0.001 df = 2). According to paired t-test Cd level from bees were higher in the industrial category than in the control category (t = 4.13, *** df = 19) and also than in the urban category (t = 3.39 ** df = 19). Bees in urban sites were more polluted with Cd than control sites (t= 2.03 * df = 19). The Pb level in bees was not statistically different in the experimental sites. The lead emission from vehicles in Finland during1994 was one ton which may be compared to 189 t in 1990 and negligible after 1994 (Melanen et al. 1999). The Oulu (site g) samples have been taken from an apiary near an airport (less than 1 km), very close to a road, which may explain the higher amount of Pb in bees in this site. The Zn level in bees was significantly different in the three categories (F = 4.57 P = 0.014). According to the paired t-test, Zn level in bees in industrial category was higher (t = 4.7, *** df = 19) but not significantly different than in the urban category (p = 0.08). Bees in urban areas were more polluted with Zn than control sites (t = 2.14 * df = 19). The Cu level in bees was significantly different in the three experimental categories (F = 6.87, P = 0.002). The paired t-test showed that the Cu level in bees in the industrial sites was higher than control sites (t = 4.96 *** df = 19) and urban sites (t = 3.53 ** df = 19). Bees in urban sites were more polluted with Cu than control sites (t =2.65 * df = 19). The Fe level in bees was significantly different in the experimental categories (F = 5.76, P = 0.005). The paired t-test showed that the Fe level from bees in the industrial sites was higher than control sites (t = 3.69 ** df = 19) but not significantly different than urban sites (p = 0.46). Bees in urban sites were more polluted with Fe than control sites (t = 2.61 * df = 19). According to the ANOVA , Mn level in bees was not significantly different in the experimental categories. There were significant correlations between the concentrations of zink and copper, zink and cadmium, and between copper and cadmium. Polish standard permits 0.4 – 0.5:g g^{-1} Pb, 0.1:g g^{-1} Cd and 10:g g^{-1}

Cu in honey or pollen (Jablonski *et al.* 1995). The accepted level of heavy metals in Finland for other food materials than those specified in the norm are 0.3:g g^{-1} of Pb, 0.1:g g^{-1} of Cd, 10:g g^{-1} of Cu and 50:g g^{-1} of Zn in food stuff. These levels may be appropriate for honey and other hive products. Although Müller and Agthe (1988) considered pollen suitable for monitoring metal levels in the environment, it does not seem possible to use honey or pollen as indicators of metal pollution in Finland. It was concluded that under Finnish conditions, honey and pollen are not good bio-indicators of heavy metal pollution. Bees are good bio-indicators of industrial and urban heavy metal pollution, especially to indicate Cd, Cu, Zn, Fe, and may be Pb. The average level of Cd, Cu, Zn and Fe that bees posses in industrial sites were 0.423:g g^{-1}, 19:g g^{-1}, 76.25:g g^{-1} and 172.5:g g^{-1} respectively. In case of Pb and Mn larger emission sites should be considered for better evaluation of bees as bio-indicators of these metals.

Table 5. Metal concentrations (:g g-1 of dry weight; arithmetic means ± SD) in bees at our sampling sites "a" – "l" (see Figure 1). Industrial, urban and control sites are indicated with, (I), (U) and asterisks (*) respectively

Site	Fe	Mn	Cu	Zn	Cd	Pb
a (I)	160 ± 19	220 ± 86	19 ± 4.8	77 ± 10	0.32 ± 0.16	0.49 ± 0.31
b (I)	260 ± 61	500 ± 170	27 ± 8.0	101 ± 14	1.2 ± 0.51	0.72 ± 0.44
d (I)	140 ± 15	100 ± 65	16 ± 3.5	68 ± 6.7	0.12 ± 0.12	0.44 ± 0.16
j (I)	130 ± 26	44 ± 20	14 ± 2.8	59 ± 10	0.05 ± 0.01	0.48 ± 0.28
c (U)	150 ± 40	530 ± 440	16 ± 1.0	75 ± 19	0.17 ± 0.11	0.33 ± 0.12
e (U)	410 ±170	180 ± 55	17 ± 5.0	81 ± 22	0.24 ± 0.11	0.60 ± 0.26
g (U)	180 ± 58	94 ± 65	17 ± 2.2	67 ± 14	0.08 ± 0.04	1.5 ± 2.5
l (U)	230 ± 44	82 ± 8.2	14 ± 0.48	61 ± 1.9	0.10 ± 0.01	0.27 ± 0.05
f *	140 ± 27	120 ± 55	14 ± 1.8	72 ± 4.7	0.13 ± 0.06	0.59 ± 0.19
h *	140 ± 55	158 ± 62	15 ± 3.0	70 ± 5.0	0.05 ± 0.02	0.58 ± 0.25
i *	130 ± 39	290 ± 160	13 ± 3.1	55 ± 11	0.18 ± 0.09	0.62 ± 0.26
k *	100 ± 19	49 ± 26	14 ± 2.4	56 ± 12	0.03 ± 0.01	0.62 ± 0.72

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