Usefulness of bee bread and capped brood for the assessment of monocyclic aromatic hydrocarbon levels in the environment

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1. Introduction

Anthropogenic pollution of the environment is a growing problem globally, and has been since the Industrial Revolution. Direct measurement of environmental pollutants can show how contaminated the soil, air, or water is, but to assess how this contamination can affect the ecosystem requires a somewhat different approach. Animal- or plant-derived samples can indicate the uptake of pollutants from anthropogenic environmental sources. Various aquatic and terrestrial organisms are used for such monitoring purposes, as are honey bees (Apis mellifera) (Bromenshenk et al., 1985; Bargańska et al., 2015). The honey bee as a species has been managed for thousands of years throughout human history, and due to its economic and agricultural importance is nowadays widespread and abundant on almost all continents. Honey bees’ worldwide distribution allows scientists to use them in various ecosystems for environmental monitoring. The bees themselves, as well as their products — honey or pollen — can be used to monitor the environment for the distribution of various pollutants: heavy metals (Bromenshenk et al., 1991; Conti and Botré, 2001; Satta et al., 2012), essential metals (Dzugen et al., 2017) radioactive substances (Haarmann, 1997), non-organic substances (Ponikvar et al., 2005), pesticides (Chauzat et al., 2006), organic contaminants like polychlorinated biphenyls (Anderson and Wojtas, 1986), and, lately, aromatic hydrocarbons (Dobrinas et al., 2008; Perugini et al., 2009). Various sampling techniques and methods are known to collect, measure, and analyse pollution
levels based on bee bodies, body parts, or bee product samples. Although honey bees are studied in detail and sampling methods are becoming more and more sophisticated (Bargànska et al., 2015), biological samples may significantly differ in pollutant accumulation, showing a wide range of pollution levels even in the same site and environment. Especially honey was well studied due to its importance as a bee product. It was found, that variation in trace element content in honey is first of all due to botanical origin rather than environmental exposition to pollution (Bogdanov 2006; Bogdanov et al., 2007), however also apiculture practices and honey processing should be considered when analysing metal content of honey samples (Pohl, 2009).

Honey bees feed on honey — which is made from floral nectar or honeydew (sap excreted by aphids living on plant sap) collected by the bees — and on pollen, also collected by worker bees from flower anthers. Floral nectar is a substance produced by the nectaries; due to the structure of flowers it is less exposed to airborne pollution and is the least polluted bee product (Formicki et al., 2013; Jovetić et al., 2018; Matin et al., 2016). Nectar can also evaporate from the flower in high temperatures or be washed out during heavy rains, which means that the plant must constantly renew it until the flower is pollinated. For this reason, honey is usually less exposed to airborne pollutants (often travelling on PM present in the air, eg. heavy metals), and contains lower levels of such pollution than honeydew or pollen, which can be exposed to airborne pollutants for longer periods of time (Maragou et al., 2017). In addition, the high viscosity of honeydew and pollen causes them to accumulate larger amounts of pollution. Nectar and pollen can be contaminated not only by the deposition of atmospheric pollution on plants, but also by plants’ uptake of pollutants like radionuclides or heavy metals from the soil (Bunzl et al., 1988; Ismael et al., 2019; Silva et al., 2012).

The presence of pollutants in honey can therefore vary based on the flower’s ability (its shape or environmental conditions during flowering) to collect these airborne pollutants, and on the plants’ ability to uptake and excrete pollutants into the produced nectar (Bunzl et al., 1988). These differences can be quite significant. For example, the level of heavy metal pollution can differ by a factor of one hundred depending on the type of sampled honey, usually reaching higher levels in honeydew than in monofloral honeys (Dzugan et al., 2017). A similar tendency was found when comparing radionuclide levels in various nectar honeys and honey from honeydew (Barisić et al., 1999). Monofloral honeys can also differ in the level of pollutants, e.g. due to differences in flower structure and shape, such as open or closed flowers or flowers standing upright or hanging down. The fresh nectar collected by bees is also mechanically filtered by the proventriculus before reaching the crop (the nectar-collecting organ of bees) and particles 100 μm or larger are caught between the stylets of the mouthparts and are not ingested (Peng and Marston, 1986). In honey, the composition of minerals or pollutants besides the raw material from which honey was produced (botanical origin) may also depend on the climatic conditions and geographical area (Bogdanov, 2006; Bogdanov et al., 2007) and was found to be a poor bioindicator of heavy metal pollution in the environment (Conti et al., 2018; Pohl, 2009; Satta et al., 2012), but also honey processing itself can cause additional contamination of collected honey (Pohl, 2009). Nevertheless, there are studies suggesting, that honey samples can indicate of the sampled honey’s geographical and botanical origins, as well as types, source, and degree of contamination (Solayman et al., 2015). Yet, having in mind the large effect of botanical and climatic conditions on pollutant uptake by honey, it is the least reliable bee product for monitoring purposes.

Pollen is usually found to be more contaminated due to its being exposed to airborne pollution longer than the continuously produced nectar, and because pollen is highly lipophilic, containing 4%–8% lipids, but in some cases as much as 22.4% (Szczesna, 2006). Honey, on the other hand, contains water, sugars, amino acids, organic acids, minerals, and other relatively hydrophilic constituents (da Silva et al., 2016). Similarly to pollen, propolis (resinous exudates gathered mainly from buds, but also from leaves, branches, and barks and mixed with the secretion of bees’ mandibular gland) also contains more contaminants (Matin et al., 2016) and various microelements than does honey (Maragou et al., 2017). However, collecting propolis from the hive is a more time-consuming and complicated procedure than collecting pollen, as the latter can be collected by using readily available pollen traps or by simply taking samples straight from a comb filled with bee bread (pollen gathered and slightly fermented for storage in cells). Therefore, bee bread can be an easily accessible bee product to be used for environmental monitoring.

For the last more than 50 years numerous studies have showed that measuring pollutant level in adult honey bees can also serve for monitoring purposes (Bromschenken et al., 1983; Crane, 1984; Conti and Botre, 2001; Wallwork-Barber et al., 1982). Most studies used adult honey bee bodies to monitor the environment for pollutants first of all. The level of pollution in bee bodies was found to be significantly different between environments and to correspond to the varying level of pollution (Bargànska et al., 2015). Heavy metal concentration in the body of adult bees was, for example, almost one hundred times larger in bees living in areas with a higher probability of pollution (Bromschenken et al., 1985). The area of foraging activity associated with honey bee colonies can generally extend over a 10-km radius (Visscher and Seeley, 1982) around the colony; however, in the natural environment bees will fly up to approximately 1.7 km (Waddington et al., 1994), while in an urban environment it is usually about 1.2 km (Garbuzov et al., 2015). These differences in foraging distance are usually due to differences in food source availability: the more diverse and rich the actual food source around the colony is, the shorter distance a bee will fly while foraging (Schneider and McNally, 1993; Beekman and Ratnieks, 2000). Nevertheless, colonies even in the same place can differ in their actual foraging area, foraging activity, distance covered, and even in preferred food sources (both for nectar and pollen). These differences between colonies can affect the amount of pollution found in the collected pollen and in bee bodies.

Bees of various age may also differ in the levels of contamination found in their body or tissues. After hatching from its egg, a bee larva is fed a high-protein diet based on pollen and royal jelly (a secretion of nursing bee glands) which is necessary for its development. Depending on its future caste, the bee larva is fed continuously for 5–8 days by the nursing bees; after consuming all the food it is provided with, it will produce a cocoon, go through metamorphosis, and finish its development. During its first few days of life after eclosion, a young bee will clean the brood cells, build up its protein level by consuming more and more pollen, and feed the older larvae. Later, when its hypopharyngeal glands are fully developed, it will also feed the younger larvae royal jelly (HAYDÁK, 1963). During the last two phases, the nursing bee may be exposed to relatively high levels of contamination present in the pollen and nectar from which it produces royal jelly for the larvae and covers its own energetic and metabolic needs for survival. Later, when hive bees turn into foragers, their diet changes: they reduce their fat bodies and consume mostly honey instead of pollen (HAYDÁK, 1963).

Monitoring airborne pollutants, like aromatic hydrocarbons, are raising more and more concerns recently. Although in the last few years the emission of air pollutants in Europe has followed a
downward trend, in Poland PM2.5 and PM10 levels continue to be among the highest rates in the EU. Reports of the World Health Organization (WHO) indicate that more than half of the 50 smog-affected European cities are located in southern Poland (WHO, 2016), a region which is both highly urbanised and industrialised. The city of Kraków is ranked 11th among them. Several factors can be the source of such high concentrations of air pollution in the capital of the Lesser Poland Voivodship. The most important one is called “low emission” (emission from sources located at a height of up to 40 m) and results from the combustion of solid fuels (e.g. low quality coal) and rubbish in heating furnaces (Burchart-Korol et al., 2016; Dzikuc and Adamczyk, 2015). Another factor affecting the quality of the air in Kraków is pollution due to vehicular traffic, in particular car exhaust fumes (Dzikuc et al., 2017). The data from 2016 presented in the TomTom Traffic Index report indicate that Kraków ranks 8th in the most congested cities in Europe, and its position in this ranking is rising. In addition, as indicated by the Statistical Yearbook of Poland (GUS, 2018), one in five cars registered in Poland is over 15 years old with an inefficient or worn-out catalytic converter. The air quality in Kraków is also strongly influenced by the location of the city in the Vistula River valley, and its dense urban development significantly limits the movement of air masses, making it impossible to disperse persistent pollution over a large area (Oleniacz et al., 2016). In the vicinity of Kraków, there are other significantly polluted areas. Stretching westward from Kraków is the Katowice industrial region, with a number of coal and ore mines, smelters, and other heavy industrial activities. One of the closest industrial sites is just outside the city of Olkusz, 30 km northwest of Kraków: the zinc smelter ZGH “Bolesław” in Bukowno. It pollutes the environment both with the by-products of previous mining activities, stored in ore heaps, and with air pollution from combustion processes and other technological processes during the production of various forms of zinc from metalliferous ores.

In our study, we described the aromatic hydrocarbon pollution levels of honey bee capped brood (larvae after finishing feeding and being in the cocoon in a closed, capped cell and later changing into pupa) and bee bread in hives (pollen collected by and prepared for storage and larval feeding by bees) located on sites with different sources of air pollution (mostly urban or mostly industrial) in southern Poland — in the city of Kraków and around the city of Olkusz. The aim of our study was to test how high the uptake in bee bread and capped brood is and how reliable single bee bread or capped brood samples are for monitoring urban and industrial areas for aromatic hydrocarbon pollution. So far, only a few studies were conducted for monitoring of polycyclic aromatic hydrocarbons (PAHs) with use of either adult honey bees (Perugini et al., 2009) or additionally honey bees products (Lambert et al., 2012; Kargar et al., 2017).

We have chosen capped brood instead of adult bees, based on the growth and life cycle of honeybees. One can except high levels of contamination in bee bodies during the last phase of larval development (Haydak, 1963). The pollution level is mostly dependent on the level of contamination of pollen, the protein source for developing larvae, therefore, capped brood may serve as a better indicator of environmental pollution levels than adult forager bees feeding mostly on nectar. Bee bread stored in the hive may also have higher pollution levels than pollen. Pollen is transported by forager bees to the hive, where it is deposited and processed for storage possibly gathering also additional pollution from the foragers’ body during deposition. Therefore bee bread may give a better picture of the total environmental pollution in the area surrounding the hive.

2. Materials and methods

Samples of bee bread (further called pollen) and capped brood were collected on two urban-type sites in the city of Kraków (sites K1 and K2, further called urban sites) and on two industrial sites near and in the city of Olkusz (sites O1 and O2, further called industrial sites). Site K1 (50°03’39.4”N 19°52’17.0”E) was located in the vicinity of the Wolski Forest in the outskirts of Kraków, 4 km from the centre, the Main Market Square, in a predominantly residential area with detached houses, meadows, and woodland, but within a few hundred metres of a public road with heavy traffic. Site K2 (50°03’40.9”N 19°55’48.1”E) was located in the old city centre of Kraków, a few hundred metres from the Main Square, in the Monastery of Minor Capuchin Friar. Considering the average flight range of honeybees, which in an urban environment can reach about 1.2 km (Garbuzov et al., 2015), the possible foraging areas of the sampled bee colonies from sites K1 and K2 did not overlap, as shown in Fig. 1, but both were located inside the city area. Site O1 (50°17’03.6”N 19°26’57.5”E) was located in the village of Bukowno, which neighbours the city of Olkusz, less than two km from the ZGH “Boleslaw” zinc smelter and the ore heaps located just outside of the city of Olkusz. Site O2 (50°18’14.6”N 19°32’34.7”E) was located at a distance of about 3 km from Olkusz’s city centre, in an area of detached houses neighbouring a woodland and lying approximately 4 km from the smelter and the ore heaps. Both sites, O1 and O2, were located approximately 3 km from national road No. 94, which experiences heavy traffic. The locations of all sample sites are presented in the maps in Fig. 1.

The pollution of atmosphere with benzene in the Lesser Poland Voivodship was monitored in 2018 and the mean concentration did not exceed 3 µg/m³. Specifically for Kraków the pollution levels with benzene for this period based on 3 sampling points were: min. 2.1 µg/m³ max. 2.8 µg/m³ and mean 2.32 µg/m³ in 2018. no such data is available for the city or the surroundings of Olkusz.

At each site, capped brood and pollen were collected from three stationary hives owned by local beekeepers. Two pieces of comb were cut out for testing, each with a surface area of at least 15 cm × 15 cm (or more when necessary) — one containing stored bee pollen and the other capped brood. Samples were collected in two seasons: 2017 and 2018. In each season, all colonies were sampled twice, in the spring (the end of April or May, depending on the weather) and in the summer (June). The pieces of comb were not dried before the analysis, due to possible losses of volatile organic compounds (BTEX), with use of either adult honey bees (Perugini et al., 2009) or additionally honey bees products (Lambert et al., 2012; Kargar et al., 2017).

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sampling time 0.33 min. The temperatures of the ion source and the quadrupole were 230 °C and 150 °C, respectively. Detection was conducted using electron impact ionization at 70 eV in selected ion monitoring (SIM) mode at an m/z of 78, 91, 106, 106, and 134 amu for selective detection and quantification of benzene, toluene, ethylbenzene, p-xylene, and n-butylbenzene, respectively.

Unfortunately, no certified reference materials are available for honey bees and pollen or bee bread therefore we used the following hydrocarbon standards: benzene, toluene, ethylbenzene, p-xylene. Their LOD values were the following: benzene 0.243 ng/g and 0.6967 ng/g, toluene 0.189 ng/g and 0.866 ng/g, ethylbenzene 0.170 ng/g and 0.943 ng/g and p-xylene 0.766 ng/g and 0.420 ng/g for capped brood and pollen, respectively. Their LOQ values were the following: benzene 0.810 ng/g and 2.322 ng/g, toluene 0.631 ng/g and 2.885 ng/g, ethylbenzene 0.566 ng/g and 3.14 ng/g and p-xylene 2.553 ng/g and 1.399 ng/g for honeybees and bee bread, respectively. Hydrocarbon concentrations in standard solutions were in the range of 0.167–0.850 mg/ml (hydrocarbon dissolved in methanol). The concentration of solution No. 7 was about 5 times higher than solution No. 6. Solution No. 7 was prepared to check whether the straightness of the calibration curve (y = ax + b) was maintained at much higher concentrations.

Calibration of the chromatographic system for the analysis of monocylic aromatic hydrocarbons was performed not for pure standard solutions, but for weights of capped brood and pollen with the addition of these solutions. This treatment was aimed at eliminating the influence of interference effects of matrix components on the course of the calibration curve.

Capped brood and pollen used for calibration were obtained from an apiary located outside Kraków. They were packed into sealed bags immediately after being removed from the patch. After packing, the material was homogenised. The homogenization time was 2 min. 14 samples of capped brood and 14 samples of pollen (0.4 g each) were prepared in glass vials. Then, each of the seven calibration solutions was dosed to two bee samples and two pollen samples in an amount of 50 µL. The amounts of individual hydrocarbons added to the samples equaled from 8.4 to 212.6 ng, which, based on 1 g of capped brood or pollen, gives values from 21 to 532 ng.

The weight of 0.4 g was the maximum mass of capped brood that could be obtained for one measurement from the average slice obtained for testing. Obtaining a smaller amount of sample would result in less volatile compounds evaporated to a larger volume of the headspace phase in the vial, and thus smaller peak areas and greater uncertainty in the results obtained. Despite weighing the same capped brood and pollen weight (0.4000 ± 0.0010 g) each time, these weights differ in volume, and thus the volume of the headspace phase in the vial (and the concentration of volatile substances in this phase) is different for each weighing. The addition of an internal standard to the weighing each time and taking into account its peak area in the calculations allows to eliminate differences in the peak areas resulting from unequal volumes of the supra-surface phase.

Therefore, in addition to calibration solutions, 43 ng of internal standard (n-butylbenzene) in the form of a 50 µL methanol solution was added to the capped brood and pollen weights, and the vial prepared in this way was sealed and subjected to chromatographic analysis.

Linearity range for each hydrocarbon, for capped brood and pollen was 0.5 µg/g. Accuracy (and precision given as standard deviation) of actual value was 137.33 (±35.78)% and 126.76 (±20.71)% for benzene, 140.05 (±27.34)% and 149.07 (±39.42)% for toluene, 101.27 (±12.48)% and 112.53 (±15.26)% for ethylbenzene and 107.61 (±14.33)% and 117.79 (±17.50)% for p-xylene for capped brood.
For the calculation of hydrocarbon concentrations in real samples, calibration curve equations in the form \( y = ax \) were used. The calibration curves were plotted as the \( S_{\text{cor}} \) (corrected area) dependence on \( c \) (hydrocarbon content per gram of capped brood or pollen). \( S_{\text{cor}} \) was calculated as the quotient of the peak area characteristic of a given hydrocarbon and the peak area of the internal standard (n-butylbenzene) (Fig. 2).

For each colony, the level of pollen and capped brood pollution were counted based on a maximum of three repeated measurements per sample. We calculated the coefficients of variance between colonies from the same site at the same time in the season and in the same year. Then, we compared these values to the coefficient of variance between sites of the same type and from the same year using a \( t \)-test. The possible correlation between pollen and capped brood samples originating from the same families at the same time were compared using Spearman’s correlation, which is less sensitive to possible strong outliers.

Next, the mean values for all families per site were counted to assess pollution levels for each site. Due to the non-normal distribution of the data, non-parametric tests were used for further analysis. For comparison of pollen and capped brood contamination levels between years and site types, mean values counted for each site were used (to weigh against unequal colony number on some sites) and analysed using Mann–Whitney’s \( U \) test. To compare pollen and capped brood contamination from the same family and to assess possible seasonal differences between sites, a \( t \)-test was used for paired comparison. All calculations were done using Statistica 13 (Dell Statistica, 2016).

3. Results

We collected 43 pollen samples and 46 samples of capped brood from 12 hives, during the two years and during two different seasons (Table 1A and B). The level of mean BTEX pollution was generally higher in the pollen (mean ± SE: 29.2 ± 2.15 \( \mu \)g/kg) than in the capped brood (17.3 ± 1.22 \( \mu \)g/kg) \( t = 21, df = 16, p = 0.015 \), although in some cases in 2017, due to high ethylbenzene levels in some samples, this trend was reversed (Table 1A). An analysis based on the mean pollution levels calculated for each colony showed that the contamination of bee bread and capped brood with BTEX on urban and industrial sites were similar (bee bread: \( U = 30, p = 0.878 \); capped brood: \( U = 29, p = 0.798 \)) (Fig. 3). However, some differences between the study years were found. The pollution levels found in the bee bread were higher in 2018 than in 2017 (\( U = 6.0, p = 0.005 \)), but not in capped brood (\( U = 30.0, p = 0.878 \)) (Fig. 4). The mean BTEX contamination of bee bread samples between spring (mean ± SE: 27.0 ± 2.95 \( \mu \)g/kg) and summer (31.4 ± 3.11 \( \mu \)g/kg) sampling were similar (\( t = -1.23, df = 19, p = 0.234 \)) and the contamination of capped brood samples were the same between seasons (spring mean ± SE: 16.8 ± 1.46 \( \mu \)g/kg and summer 17.8 ± 1.99 \( \mu \)g/kg) (\( t = -0.35, df = 21, p = 0.729 \)).

Detailed analysis of the four measured components of BTEX contamination did not show any significant difference between the
significantly different between the spring and summer samples of bee bread and the capped brood, except for toluene levels in the bee bread. The summer samples had significantly higher toluene levels in the bee bread (Table 2B).

The benzene ($r(40) = 0.423$, $p = 0.005$), toluene ($r(40) = 0.604$, $p < 0.001$), and p-xylene ($r(40) = 0.561$, $p < 0.001$) levels in the bee bread and the capped brood, except for toluene levels in the bee bread. The summer samples had significantly higher toluene levels in the bee bread (Table 2B).

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### Table 1
Mean pollution (±SD) levels with BTEX on two industrial and two urban sites measured twice during the season: during spring and during the summer in bee bread stored by bees and in capped brood and in two consecutive seasons: 2017 (A) and 2018 (B).

#### A) Urban sites

|                  | K1 spring Bee bread | K1 summer Bee bread | K2 spring Bee bread | K2 summer Bee bread |
|------------------|---------------------|---------------------|---------------------|---------------------|
| Benzene          | 0.4 ± 0.02          | 0.1 ± 0.05          | 0.7 ± 0.23          | 0.1 ± 0.07          |
| Toluene          | 3.2 ± 0.28          | 2.5 ± 0.84          | 14.4 ± 1.42         | 6.1 ± 2.31          |
| Ethylbenzene     | 0.4 ± 0.03          | 3.6 ± 1.39          | 1.1 ± 0.13          | 5.3 ± 2.55          |
| p-Xylene         | 5.1 ± 0.17          | 4.2 ± 1.27          | 23.5 ± 4.23         | 13.5 ± 5.47         |
| Sum of all pollutants (BTEX) | 9.0 ± 0.49                  | 10.5 ± 3.52                  | 39.8 ± 5.7               | 25.1 ± 10.3           |
| Proportion of BTEX in pupa compared to pollen | 116.0% | 63.0% | 21.4 ± 2.69 | 8.0 ± 2.23 |

#### B) Industrial sites

|                  | O1 spring Bee bread | O1 summer Bee bread | O2 spring Bee bread | O2 summer Bee bread |
|------------------|---------------------|---------------------|---------------------|---------------------|
| Benzene          | 0.3 ± 0.00          | 0.2 ± 0.01          | 0.2 ± 0.07          | 0.6 ± 0.3           |
| Toluene          | 4.0 ± 0.00          | 4.0 ± 0.4           | 6.5 ± 0.8           | 12.3 ± 6.51         |
| Ethylbenzene     | 0.3 ± 0.00          | 5.6 ± 2.86          | 0.5 ± 0.09          | 9.0 ± 0.77          |
| p-Xylene         | 4.3 ± 0.00          | 5.9 ± 1.34          | 7.8 ± 0.34          | 11 ± 3.14           |
| Sum of all pollutants (BTEX) | 8.9 ± 0.00                  | 15.7 ± 4.58                  | 14.9 ± 1.3           | 32.9 ± 10.62         |
| Proportion of BTEX in pupa compared to pollen | 177.1% | 220.8% | 21.4 ± 2.69 | 8.0 ± 2.23 |

### Fig. 3
Mean (±SE) BTEX (sum of benzene, toluene, ethylbenzene and p-xylene) levels (µg/kg) in samples of bee bread and capped brood, collected on urban and industrial sites in Poland.

### Fig. 4
Mean (±SE) BTEX (sum of benzene, toluene, ethylbenzene and p-xylene) levels (µg/kg) in samples of bee bread and capped brood collected in Poland during two sampling seasons.
capped brood corresponded positively to the pollution levels found in bee bread, while ethylbenzene showed a somewhat weaker and negative correlation ($r(40) = -0.350$, $p = 0.023$) between the two sample types (Fig. 6).

The coefficients of variance between the samples from the same site and the same season were compared, and we found that in the case of bee bread these values ranged between 2.6% and 59.2% per site (Table 3A), while in the case of the bee capped brood the samples ranged between 9.6% and 41.5% (Table 3B). No significant difference was found between the coefficients of variance for the bee bread and capped brood samples ($t = -0.46$, $df = 15$, $p = 0.652$).

We also calculated the coefficient of variance between sites and found similar or somewhat higher values as the coefficient of variance between colonies at the same site (Table 3). For bee bread, the range was between 6.9% and 76.6% (Table 3A), while for capped brood it was 12.7%–71.6% (Table 3B).

4. Discussion

Our results show that the environmental monitoring of BTEX can be based on sampling honey bee capped brood, and bee bread in particular. However, there is a significant difference in the uptake of these pollutants regarding sample type. Bee bread collected as a food source revealed consistently higher levels of BTEX than capped brood (Fig. 3), as well as differences between years, as opposed to capped brood.

Honey bees in urban areas collect pollen from 0.5 to 1.2 km around the hive (Garbuzov et al., 2015). However, this 0.8–4.5 km² area in the case of urban honey bees may not be covered uniformly by foragers of each family. Bees learn the location of a food source from each other, so each colony might forage on different areas of this larger potential zone. Such foraging differences can result in varying pollution uptake depending on where in the surrounding area and on which flowers bees of a certain colony mostly forage. These differences are quite visible when comparing the coefficient of variance between colonies from the same site. Both bee bread and capped brood samples showed a wide variance, suggesting that the families studied indeed used different food sources, even when located on the same sites (Table 1). Actually, the coefficient of variance between colonies from the same site and the coefficient of variance between mean pollution levels of the four various sites are similar. The wide variance of pollution levels measured at the same site but in different hives shows clearly that monitoring should be based on more than one colony (Table 3). A minimum of three colonies, like in our study, or optimally more, should be used to achieve an accurate mean pollution level per site. This is even more true if only small differences are expected in pollution levels between sites, like in our example. Various studies used so far different number of colonies for biomonitoring purposes. Some based their results on a single colony per site, others used more, usually at least three colonies per site.

Honey bee larvae are fed primarily royal jelly with a growing addition of pollen over time. Pollen (both in the form of royal jelly
and as bee bread) is the larvae’s source of the proteins necessary for development. Lower pollution levels found in honey bee capped brood are a natural phenomenon and corresponds to the results of Lambert et al. (2012) who also found lower levels of PAHs in honey bee bodies, than in pollen samples on the same sites. In addition to protein (from pollen) bees also need carbohydrates, lipids, minerals, and water for their development, which are found mostly in the honey produced from flower nectar. Honey made from flower nectar is a less polluted food source (Formicki et al., 2013; Jovetić et al., 2018), so feeding the larvae both honey and pollen can explain the lower, more diluted pollution levels in the capped brood’s bodies.

Lower pollution levels in capped brood were not followed by lower variance levels, as one might expect. Pollution levels tend to present a left-skewed lognormal distribution, causing lower variance in the case of lower mean values, due to the skewness of data. Therefore, one can assume that bee bread — with its higher mean pollution values and similar variance between samples from the same site — actually shows a more accurate picture of pollution than the less-polluted capped brood.

Bee bread pollution levels may also more accurately correspond to environmental pollution level than capped brood. The BTEX levels found in capped brood which were fed the bee bread present in the hive and analysed for pollution did not correspond fully to the levels found in the bee bread. While in the bee bread samples, substantially more BTEX was found in 2018 in the capped brood, such increased levels of BTEX pollution did not appear in that year of our study; moreover, the capped brood actually showed similar levels on both site types and through both years, regardless of changes in bee bread pollution. There are two possible explanations: either there is a mechanism controlling some of the BTEX pollution levels in the capped brood or the nursing bees were choosing less-polluted bee bread to feed to the larvae. In both cases, monitoring of BTEX pollution in the capped brood may result in a false, reduced picture of overall pollution, due to the controlled or selective uptake of pollutants. This could explain why in our samples the levels of three out of four pollutants (benzene, toluene, and p-xylene), nevertheless, correlated between the bee bread and capped brood samples, while one (ethylbenzene) did not, and actually showed a negative correlation. Assuming possible differences in pollution levels at different time-points, there is also a possibility that in some cases the bee bread samples taken from the hive were not fresh and therefore represented a different timeframe for pollution than the larvae, which are usually fed fresh bee bread. Such a scenario could also cause not only discrepancies between the pollution levels found in the capped brood and bee bread samples, but higher pollution levels in the capped brood than in the bee bread taken from the same colony at the same time. Such a
situatio also occurred in our study at site O1, during both the spring and the summer sampling, and in the case of the spring samples from K1 and K2 in 2017 (Table 1). In the case of the O1 and K1 spring samples, in some of the colonies the amount of bee bread was actually too scarce to run the analysis; therefore, the results obtained from the bee bread are based on one or two colonies. This could also mean that the nursing bees in these colonies were forced probably leftover, older bee bread in the hive to feed the sampled capped brood during open brood phase. As a result, they could have fed them bee bread which was more polluted, as it would have been collected earlier in the season when household heating was still causing more air pollution on these sites. Although in our case it might cause a discrepancy, yet sampling bee bread on marked combs (combs added at known dates and their filling up controlled) can allow for sampling pollutants for longer periods of time. Additionally bee bread sampling will not deprive the colony of fresh pollen completely, like in case of using pollen traps. Based on our results, we suggest that for measuring and monitoring of BTEX pollution the use of bee bread is a better source of information about environmental pollution levels than capped brood. Bee bread usually has a higher level of pollution than capped brood, which allows for more accurate analysis, and it is also easier to extract from the cell than capped brood. Bee larvae may also be fed selectively or may possess a mechanism which controls the uptake of BTEX from food. It is also important to remember that honey bee families — even if they are located in the same place — can prefer certain areas and pollen sources (flowers). Therefore, sampling should be based on a minimum of three, but ideally even more, bee families in order to have a better coverage of the tested area.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**CRediT authorship contribution statement**

**Katarzyna Zieba:** Methodology, Formal analysis, Investigation, Writing - original draft. **Elżbieta Szostak:** Formal analysis, Writing - original draft. **Krystyna Czekowska:** Methodology, Investigation, Writing - original draft. **Paweł Miskowicz:** Methodology, Formal analysis, Investigation, Writing - original draft. **Agnieszka Moos-Matysik:** Formal analysis, Investigation, Writing - original draft. **Anna Nyczyk-Malinowska:** Investigation, Writing - original draft, Funding acquisition, Supervision.

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**Table 3**

Coefficient of variance of BTEX pollution levels between colonies on the same sites and between sites in bee bread (A) and capped brood samples (B).

| Year | Area | Season | Site | Colonies with bee bread samples | Coefficient of variance between colonies | Sites with bee bread samples | Coefficient of variance between sites |
|------|------|--------|------|---------------------------------|------------------------------------------|-------------------------------|--------------------------------------|
| 2017 | Urban| Spring | K1   | 2                               | 5.4                                      |                               |                                      |
| 2017 | Urban| Spring | K2   | 3                               | 59.2                                     |                               |                                      |
| 2017 | Industrial | Spring | O1   | 1                               |                                          |                               |                                      |
| 2017 | Industrial | Spring | O2   | 3                               | 12.6                                     | 3                             | 46.7                                |
| 2017 | Urban| Summer | K1   | 3                               | 14.3                                     |                               |                                      |
| 2017 | Urban| Summer | K2   | 3                               | 37.1                                     |                               |                                      |
| 2017 | Industrial | Summer | O1   | 3                               | 8.7                                      |                               |                                      |
| 2017 | Industrial | Summer | O2   | 2                               | 55.2                                     | 4                             | 76.6                                |
| 2018 | Urban| Spring | K1   | 3                               | 41.0                                     |                               |                                      |
| 2018 | Urban| Spring | K2   | 3                               | 26.8                                     |                               |                                      |
| 2018 | Industrial | Spring | O1   | 3                               | 40.6                                     |                               |                                      |
| 2018 | Industrial | Spring | O2   | 3                               | 45.4                                     | 4                             | 6.9                                 |
| 2018 | Urban| Summer | K1   | 3                               | 2.6                                      |                               |                                      |
| 2018 | Urban| Summer | K2   | 3                               | 26.9                                     |                               |                                      |
| 2018 | Industrial | Summer | O1   | 3                               | 13.7                                     |                               |                                      |
| 2018 | Industrial | Summer | O2   | 3                               | 12.3                                     | 4                             | 14.9                                |

| Year | Area | Season | Site | Colonies with capped brood samples | Coefficient of variance per colony | Colonies with capped brood samples | Coefficient of variance per colony |
|------|------|--------|------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| 2017 | Urban| Spring | K1   | 3                                 | 33.6                                | 3                                 | 46.5                                |
| 2017 | Urban| Spring | K2   | 3                                 | 12.3                                | 3                                 | 46.5                                |
| 2017 | Industrial | Spring | O1   | 3                                 | 29.2                                | 4                                 | 71.6                                |
| 2017 | Industrial | Spring | O2   | 3                                 | 27.8                                | 4                                 | 71.6                                |
| 2017 | Urban| Summer | K1   | 3                                 | 41.1                                | 4                                 | 71.6                                |
| 2017 | Urban| Summer | K2   | 3                                 | 13.3                                | 4                                 | 71.6                                |
| 2017 | Industrial | Summer | O1   | 3                                 | 32.3                                | 4                                 | 71.6                                |
| 2017 | Industrial | Summer | O2   | 2                                 | 31.0                                | 4                                 | 71.6                                |
| 2018 | Urban| Spring | K1   | 3                                 | 41.5                                | 4                                 | 71.6                                |
| 2018 | Urban| Spring | K2   | 3                                 | 10.3                                | 4                                 | 71.6                                |
| 2018 | Industrial | Spring | O1   | 3                                 | 13.6                                | 4                                 | 71.6                                |
| 2018 | Industrial | Spring | O2   | 3                                 | 22.3                                | 4                                 | 71.6                                |
| 2018 | Urban| Summer | K1   | 3                                 | 24.9                                | 4                                 | 71.6                                |
| 2018 | Urban| Summer | K2   | 3                                 | 9.6                                 | 4                                 | 71.6                                |
| 2018 | Industrial | Summer | O1   | 3                                 | 19.4                                | 4                                 | 71.6                                |
| 2018 | Industrial | Summer | O2   | 3                                 | 25.9                                | 4                                 | 71.6                                |
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114882.

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