Pesticides in the urban environment: Targeted and non-targeted screening of pesticide profiles in urban honey from Montreal by LC-QTOF-MS.

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Abstract

The safety of urban honey is a growing concern due to pesticide contamination, necessitating effective monitoring methods. Small-scale urban beekeeping has emerged as a promising solution to counter the honeybee population decline and high honey demand. However, urban pesticides pose risks as bees unintentionally collect them while foraging, leading to pesticide residue accumulation in honey. This issue has been substantiated by various reports confirming the occurrence of pesticides in urban settings and their detection in honey, underscoring the importance of addressing this concern for both honey producers and consumers. Recognized as a promising approach for pesticide residue monitoring in food samples, non-targeted analysis (NTA) has gained significant attention. While the application of non-targeted studies has seen an uptick, research specifically focusing on honey is limited, and as far as we know, no studies have been conducted on urban honey to enhance our understanding of human exposure to contaminants in such honey. To address these concerns, in Chapter 3, a direct injection technique coupled with high-performance liquid chromatography and quadrupole time-of-flight mass spectrometry was employed as an NTA to detect pesticides in urban honey. The technique was validated according to the SANCO guideline recommendations for 21 key pesticides, and was assessed to be robust and sensitive for this application. It was able to detect pesticide residues 2 to 1000 times below Canada's 0.1 ppm limit and yielded comparable results to methods involving prior sample preparation. The instrument linearity, repeatability, and recoveries met satisfactory criteria, ensuring the method's reliability. Subsequently, the method was applied for targeted (79 pesticides) and non-targeted screenings of 118 urban honey samples collected from Montreal, Canada in 2021. None of the 79 pesticide residues were present at levels above the Limit of Detection (LOD) using the newly developed targeted screening, suggesting that targeted pesticides are of no concern.

However, employing an NTA revealed the presence of 111 compounds tentatively identified with scores above 80%, emphasizing the need for further investigation. The study compared the physicochemical properties of urban honey with rural honey from Quebec, revealing no significant differences except for slightly higher electrical conductivity in urban honey. This suggests that relying solely on physicochemical properties is inadequate to differentiate between urban and rural honey. The developed method effectively detected pesticides in urban honey, while the NTA identified unknown compounds warranting further investigation. Additionally, the comprehensive analysis of urban honey samples, including moisture content, pH, and electrical conductivity, provided valuable insights into the quality of honey collected from Montreal in 2021. In Chapter 4, the investigation aimed to evaluate the potential of pesticide measurements in urban honey and air as mutually beneficial and supplementary approaches by comparing the results with those obtained from an artificial XAD-resin-based passive air sampler (PAS). 118 urban honey samples were collected from Montreal, while 40 sites across Montreal were selected for the deployment of the XAD-PAS over three months during the summer of 2021. The screening technique combining an NTA and HPLC-QTOF-MS was used to identify pesticides in both matrices obtained from urban areas. The method was effective in identifying targeted compounds of interest. The insect repellent DEET was found in 28 of the 40 passive air samplers analyzed. While DEET, a known environmental contaminant, was found in PASs, its presence could not be unequivocally identified in the urban honey samples. None of other target pesticides were detectable in the PAS. The findings from this study offer proof that measuring pesticides in both urban honey and air can effectively function as complementary and advantageous methods. In summary, this study showcased the effectiveness of NTA in enhancing our understanding of the presence of contaminants in urban honey.

Resumé

La sécurité du miel urbain est une préoccupation croissante en raison de la contamination par les pesticides, nécessitant des méthodes de surveillance efficaces. L'apiculture urbaine à petite échelle est une solution prometteuse contre le déclin des abeilles et la demande élevée de miel. Cependant, les pesticides urbains posent des risques car les abeilles les collectent involontairement, accumulant ainsi des résidus de pesticides dans le miel. Des rapports confirment la présence de pesticides en ville et dans le miel, soulignant l'importance de résoudre ce problème pour les producteurs et consommateurs. L'analyse non ciblée (ANC) est une approche prometteuse pour surveiller les résidus de pesticides, mais les études spécifiques sur le miel sont limitées. Jusqu'à présent, aucune étude n'a été réalisée sur le miel urbain pour comprendre l'exposition humaine aux contaminants. Pour aborder ces préoccupations, au Chapitre 3, une technique de solubilisation diluer-et-injecter avec chromatographie liquide haute performance et spectrométrie de masse quadrupôle temps-de-vol a détecté les pesticides dans le miel urbain. La technique, validée selon les directives de SANCO avec 21 pesticides, a montré une robustesse et une sensibilité. Elle a identifié des résidus de 2 à 1000 fois en dessous de la limite réglementaire canadienne de 0,1 ppm et a produit des résultats comparables aux méthodes impliquant une préparation préalable des échantillons. La linéarité de l'instrument, la reproductibilité et les récupérations répondaient à des critères satisfaisants, garantissant la fiabilité de la méthode. Par la suite, la méthode a été appliquée pour des dépistages ciblés (79 pesticides) et non ciblés de 118 échantillons de miel urbain collectés à Montréal, au Canada, en 2021. Aucun des 79 résidus de pesticides n'était présent à des niveaux supérieurs à la limite de détection (LOD) en utilisant le dépistage ciblé nouvellement développé, ce qui suggère que les pesticides ciblés ne posent aucun problème. Cependant, l'analyse non ciblée a révélé la présence de 111 composés provisoires avec des scores supérieurs à 80%, soulignant la

nécessité de poursuivre les investigations. L'étude a comparé les propriétés physico-chimiques du miel urbain avec celles du miel rural du Québec, ne révélant aucune différence significative, sauf une légèrement plus grande conductivité électrique dans le miel urbain. Cela suggère que se fier uniquement aux propriétés physico-chimiques est insuffisant pour différencier le miel urbain du miel rural. La méthode développée a efficacement détecté les pesticides dans le miel urbain, tandis que l'analyse non ciblée a identifié des composés inconnus nécessitant des investigations approfondies. De plus, l'analyse complète des échantillons, incluant la teneur en humidité, le pH et la conductivité électrique, a fourni des informations précieuses sur la qualité du miel. Au chapitre 4, l'enquête visait à évaluer le potentiel des mesures de pesticides dans le miel urbain et l'air en tant qu'approches mutuellement bénéfiques et complémentaires en comparant les résultats à ceux obtenus à partir d'un échantillonneur passif d'air (EPA) artificiel à base de résine XAD. 118 échantillons de miel urbain ont été collectés à Montréal, tandis que 40 sites à travers Montréal ont été sélectionnés pour le déploiement de l'EPA XAD pendant trois mois pendant l'été 2021. La technique de dépistage combinant une NTA et une HPLC-QTOF-MS a été utilisée pour identifier les pesticides dans les deux matrices obtenues à partir des zones urbaines. La méthode s'est avérée efficace pour identifier les composés ciblés d'intérêt. Le répulsif anti-insectes DEET a été trouvé dans 28 des 40 échantillonneurs passifs d'air analysés. Bien que le DEET, un contaminant environnemental connu, ait été trouvé dans les EPA, sa présence n'a pas pu être clairement identifiée dans les échantillons de miel urbain. De plus, aucun pesticide ciblé n'a été détecté dans les EPA en utilisant la méthode spécifique. Les conclusions de cette étude prouvent que mesurer les pesticides dans le miel urbain et l'air fonctionne efficacement comme méthodes complémentaires. En bref, cette étude montre l'efficacité de l'ANC pour mieux comprendre les contaminants dans le miel urbain.

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Contribution of authors

The thesis is written in the manuscript format and includes four chapters. The first chapter provides an introduction to the topic of urban honey and outlines the research objectives. Chapter 2 is a comprehensive literature review that delves into the details of non-targeted analysis and explores the subject of urban honey and its contaminants. Chapters 3 to 4 consist of manuscripts linked logically and sequentially through connecting paragraphs.

Dr. Stéphane Bayen, the thesis supervisor, provided direct advisory input and critically edited the manuscripts throughout the project. In Chapter 3, titled "Development and Validation of a Method for the Simultaneous Targeted and Non-Targeted Screening of Pesticides in Urban Honey," the author at hand assumed the responsibility for the conceptualization, fieldwork design, experimental procedures, laboratory tasks, data acquisition, data analysis, and manuscript preparation. Shaghig Bilamjian collaborated by providing, transferring, and managing urban honey samples. Additionally, Shaghig analyzed the physicochemical properties of rural honeys for comparison. Dr. Lei Tian and Shaghig collaborated on sample preparations, extraction, and spiking, with Dr. Tian playing a significant role in preparing standards. Furthermore, Dr. Lei Tian assisted in interpreting the data, and Dr. Shawn Chahal helped interpret the principal component analysis data. I would also like to sincerely thank Lan for their unwavering assistance and support throughout the entire project.

For Chapter 4, titled "Analysis of Passive Air Samplers and Urban Honey for Pesticides in Urban Environments," the author currently in focus undertook the tasks of conceptualizing the study, designing the fieldwork and experiments, conducting laboratory work, analyzing the data, and

preparing the manuscript. Instrument data acquisition and treatment were completed by Dr. Lan Liu. Dr. Stéphane Bayen, Shaghig Bilamjian, Dr. Geraldine Delbes, Dr. Philippe Apparicio, and Antoine Gillet all contributed to the deployment and collection of passive air samplers. Dr. Philippe Apparicio conducted the statistical analysis of the passive air samplers and provided the maps. I would like to extend my heartfelt appreciation to Frank Wania for his generous support throughout our project. His contribution in providing the deployment and retrieval of passive air samplers protocol, as well as the XAD-resin, has been invaluable, and his continuous assistance and guidance have been instrumental in the success of our research.

Conference presentations

- C. Akiki, S. Bilamjian, G. Leung , L. Liu, L.Tian, S. Bayen; Determination of contaminant profile in urban honey from Montreal using a non-targeted approach by LC-QTOF-MS. FONCER PURE annual symposium, Montreal, Canada, 20th January 2023. (Poster presentation).

- C.Akiki , S. Bilamjian, G. Leung, L. Liu, L. Tian, S. Bayen;, Determination of contaminant profile in urban honey from Montreal using a non-targeted approach by LC-QTOF-MS. 1st Scientific Symposium & 3rd Annual General Meeting, Laval, Canada, 9th December 2022. (Oral communication and poster presentation).

- L. Liu, **C. Akiki**, L. Tian, A. Gillet, X. Zhang, F. Wania, G. Delbès, P. Apparicio, and S. Bayen: Non-targeted screening of environmental contaminants in passive air sampler extracts from Montreal using LC-Q-TOF-MS. Canadian Chemistry Conference and Exhibition (CCCE). Calgary, Canada, 15th June 202. (Poster presentation)

- C. Akiki, S. Bayen; Determination of contaminants profile in urban honey from Montreal using a nontargeted analysis. METRC Spring Symposium 2022, Sainte-Anne-de-Bellevue, Canada, 4th May 2022. (Oral communication)

- C. Akiki; Comparaison des profils chimiques en perturbateurs endocriniens dans l'air extérieur et le miel urbain à Montreal par analyse chimique non ciblée. FONCER PURE annual symposium, Montreal, Canada, 10th November 2021. (Poster presentation)

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Abbreviations

ACN	Acetonitrile
AAS	Active air sampler
ANOVA	Analysis of variance
Aw	Water activity
CFIA	Canadian Food Inspection Agency
DEET	Diethyltoluamide
DMF	N-(2,4-Dimethylphenyl)formamide
DPMF	N'-(2,4-Dimethylphenyl)-N-methylformamide
EC	Electrical conductivity
ESI	Electrospray ionization
GC	Gas chromatography
HMF	5-hydroxymethylfurfural
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
IDL	Instrument detection limit
LC-MS	Liquid chromatography coupled to mass spectrometry
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LC-QTOF-MS	Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MDL	Method detection limit
ME	Matrix effect
MRL	Maximum residue limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NI	Neonicotinoids
NTA	Non-targeted analysis
PAS	Passive air samplers
PAHs	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PCDL	Personal compound database and library
POP	Persistent organic pollutant
PUF	Polyurethane foam
QA	Quality assurance

QC	Quality control
Q-TOF	Quadrupole time-of-flight
QuEChERS	"Quick, easy, cheap, effective, rugged and safe"
RE	Recovery
RF	Response factor
RSD	Relative standard deviation
RT	Retention time
S/N	Signal-to-noise ratio
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SVOC	Semi-volatile organic contaminants
VOC	Volatile organic contaminants
UHPLC	Ultrahigh-performance liquid chromatography

Chapter 1: Introduction

1.1 General introduction

Bee products, particularly honey, are widely recognized for their natural, healthy, and pure image, making them a popular choice considered by many (Bogdanov, 2005). However, the over-reliance on pesticides has resulted in environmental problems, including pesticide contamination in food, posing a potential risk to human health (Colin et al., 2004; Eissa et al., 2014; Porrini et al., 2003). Bee products may be produced in an environment vulnerable to contamination by various pollutants, including pesticides. Pesticides applied to crops can contaminate the soil, air, water, and flowers, which can in turn lead to the introduction of toxic chemicals into the food chain (Colin et al., 2004; Porrini et al., 2003).

Hives can be contaminated through direct or indirect exposure to pesticides. Direct contamination may occur when hives are treated with acaricides for example, while indirect contamination may occur when bees come into contact with pesticides while foraging within a radius of 3-6 km from the hive (Beekman & Ratnieks, 2000; Eissa et al., 2014). Bees are excellent mobile samplers and therefore bioindicators of chemical contamination because they encounter many pollutants during their foraging flights (Jovetic et al., 2018), and numerous researchers have suggested that bees and bee products can serve as biological indicators of environmental pollution in the areas where they forage (Al Alam et al., 2017; Balayiannis & Balayiannis, 2008; Ruschioni et al., 2013; Smith et al., 2021). Pesticides can impair the beneficial properties of honey and pose a risk to human health if present in large quantities (Blasco et al., 2004). Checking for pesticide residues in honey allows for an assessment of the potential health risks to consumers and provides insight into the use of pesticides in crops near the beehives (Taddia et al., 2004).

Urban apiculture has seen a surge in popularity in recent years as a result of efforts to enhance pollination, improve sustainability, food security, biodiversity in cities, and reverse the decline in the world's bee population. The assumptions are that bees are exposed to higher biodiversity, fewer pesticides in urban gardens than in standard agricultural regions, and cities are 2–3 degrees warmer than the surrounding countryside, making cities ideal habitats for bees. Despite the growing popularity of urban apiculture, little has been done to verify the quality and safety of honey produced exclusively in cities.

1.2 Research objectives

Given these concerns, the purpose of this project is to investigate the presence of contaminants, specifically pesticide residues, in honey produced in the urban Montreal region through a non-targeted analysis method. The project will specifically focus on achieving the following goals:

Aim 1: To develop and validate a technique using high-performance liquid chromatography, coupled to data independent acquisition mass spectrometry, based on a simplified approach that involves dilution prior to analysis, for the targeted and non-targeted screening of pesticides in honey.

Aim 2: To evaluate the potential of pesticide measurement in honey and air as mutually beneficial and supplementary approaches, yielding diverse information about pesticide exposure in urban contexts.

1.3 Hypotheses

1. Methods based on high-performance liquid chromatography with a direct injection technique, coupled to data-independent acquisition mass spectrometry, can detect a wide range of pesticides in honey at trace levels.

2. The measurement of pesticides in both honey and air will reveal complementary and diverse information, supporting the notion that these methods are mutually beneficial approaches for assessing pesticide exposure in urban environments.

Chapter 2: Literature Review

2.1. Urban honey

2.1.1. Honey

Honey is a naturally occurring sweet substance produced by *Apis mellifera* bees through the collection and transformation of nectar from plants or secretions from living parts of plants, or excretions from plant-sucking insects, like the sugary excretions from aphids, according to the European Union's definition. The nectar is gathered by bees, who then process, deposit, dehydrate, store, and mature it in their honeycombs (European Council, 2001). The composition and flavor of honey are dependent on the floral source from which the bee's collect nectar. Honey is a complex mixture of sugars, organic acids, phenolic compounds, vitamins, minerals, enzymes, and other bioactive substances, which have potential culinary and medicinal value (Alvarez-Suarez et al., 2010). This review aims to analyze the available information on contaminants in honey and provide an update on the current understanding of the safety of honey produced in urban environments.

2.1.2. The timeless legacy of honey: A journey through ancient history

Humans have been harvesting honey since ancient times, with evidence suggesting that honey hunting began during the Palaeolithic and Mesolithic periods (Alvarez-Suarez et al., 2010). Honey was a crucial source of concentrated sweetness and an important food for early humans since it was the only natural sweetener readily available (Alvarez-Suarez et al., 2010). The use of honey as food has a long and revered history dating back to early civilizations. In the biblical story of Samson, honey was discovered in a lion's carcass and shared with others, reflecting its prized status as a food source. The 10,000-year-old painting found at the Bicorp Cave in Spain depicting two individuals collecting honey from a bee's nest is evidence of the early relationship between humans

and honey (Allsop & Miller, 1996). Honey was widely used in medicine, including surgeries and burns, before the discovery of bacteria and fungi, and was included in nearly all Egyptian medicines (Crane, 1980). Until the 18th century, honey was the primary sweetener in Europe, harvested and sold for its therapeutic benefits (Crane, 1980).

2.1.3. The buzz about Canadian beekeeping and honey trade: An overview

Beekeeping plays a crucial role in the agricultural industry of Canada as it provides honey, hive products, and important pollination services to farmers of various crops, including orchard fruits, vegetables, and hybrid canola seeds (Government of Canada, 2020). The value of these pollination services has been estimated to be between $4 to 5.5 billion (BOMA Canada, 2019). Despite challenges posed by the COVID-19 pandemic, Canadian beekeepers in 2021 saw a historic high in the number of colonies and a 7.9% increase in honey production (Government of Canada, 2021). Canada is a significant player in the global honey industry. The Prairie Provinces in Canada are the source of 75.8% of honey exports, with Ontario and Quebec accounting for the remaining 22.9% (Government of Canada, 2021). In 2021, Canada produced 89.8 million pounds of honey, primarily from Alberta (38.9%), Saskatchewan (21.9%), and Manitoba (20.8%) (**Figure 2 *1***)(Government of Canada, 2021). The country exported 7 thousand tonnes of honey primarily to Japan (56.1%), the United States (39.3%), and South Korea, and imported an estimated 8 thousand tonnes of honey (Government of Canada, 2021). The total value of the 2021 honey harvest in Canada reached $278 million due to higher production volumes and strong market prices (Government of Canada, 2021).

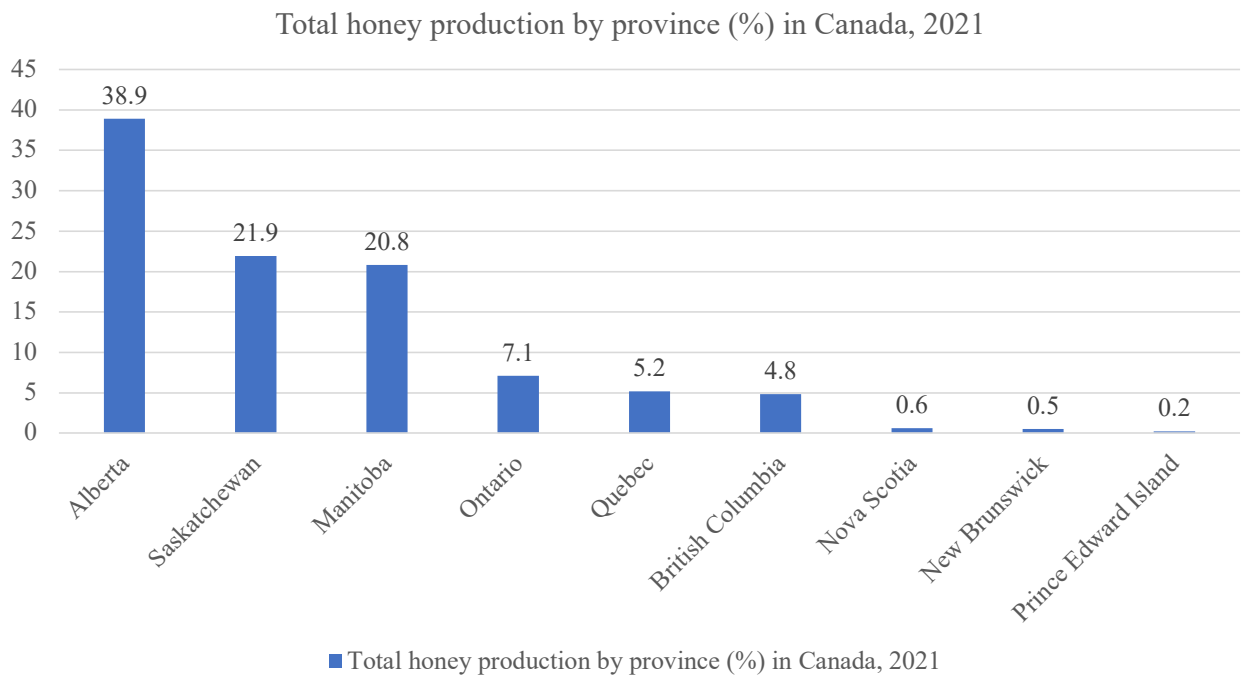

Figure 2 1 Percentage of honey production by provinces in Canada in 2021 (Government of Canada, 2021)

2.1.4. Urban beekeeping: A historical overview

Urban agriculture has become a growing trend in urban areas, which are defined as having a population density of at least 400 individuals per square kilometer and a minimum population of 1,000 (Statistics Canada, 2011). City farming has taken many forms, including community gardens and urban rooftop hydroponic vegetable production, and Montreal's community garden program, which began in 1975, was one of the first to initiate this movement (Shinewald, 2019). Urban agriculture has gained popularity globally, with notable examples such as Gotham Greens in New York and Chicago, Square Roots in New York, and Lufa Farms in Montreal ((Paschapur & Bhat, 2020; Shinewald, 2019).

Urban beekeeping has also become increasingly popular in recent years, with the number of apiarists rising in major metropolitan regions (Matsuzawa & Kohsaka, 2021). Today, urban

apiculture is practiced in numerous prominent cities worldwide, including Paris, London, Sydney, Hong Kong, Tokyo, Melbourne, Vancouver, Toronto, Montreal, New York, Quebec, and Ottawa (BOMA Canada, 2019; Shinewald, 2019). Currently, in Montreal, Alvéole, a pioneer in urban apiculture, manages nearly 868 hives across the city (Alvéole, 2022). The honeys produced by these hives contain traces of over 25 plant species including raspberry, pine, cherry, apple, and clover, as well as pollen from herbaceous plants like St. John's wort and toadflax(CULTIVE ta VILLE, 2022). According to Alvéole (2022), an urban beehive produces approximately 15 kilos of honey per year, although no clear statistics on urban honey production are available. To safeguard the bee colonies, it is necessary to register the beehives with MAPAQ, the governmental agency that regulates beehives in Montreal (Government of Canada, 2023).

2.1.5. What is honey?

2.1.5.1. Description & composition

Honey, a thick and sweet food produced by bees, is a highly nutritious source of carbohydrates that provides 3.04 kcal/g (Bradbear et al., 2009). It is primarily made up of inverted sugar, glucose, and fructose, with sugars and water making up more than 95% of its dry mass (Manyi-Loh et al., 2011). In addition, honey contains minor components such as vitamins (B6, thiamine, niacin, riboflavin, and pantothenic acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), organic acids, amino acids, flavonoids, and phenolic compounds and aromatic substances (da Silva et al., 2016). This composition, particularly the minor components, dramatically varies according to the botanical and geographical origins of the product and can alter significantly depending on storage duration and circumstances (Santos-Buelga & González-Paramás, 2017).

Honey's unique composition and chemical properties make it well-suited for long-term preservation. However, some modifications to its composition can occur over time because of various chemical and biochemical processes such as fermentation, sugar dehydration, and oxidation. These processes can cause changes in the acidity levels of the honey and lead to the formation of compounds like 5-hydroxymethylfurfural (5-HMF), which negatively impacts the quality of honey and affects its sensory properties (Santos-Buelga & González-Paramás, 2017). The following sections delve into the various compounds of honey, offering a general overview of its composition.

2.1.5.2. Sugar profile

Honey contains sugars that are important for its properties including energy, granulation, viscosity, and hygroscopicity(da Silva et al., 2016). The average sugar content in honey is 80%, mainly composed of glucose and fructose (Crane, 1980).Other sugars like sucrose, rhamnose, nigerobiose, trehalose, isomaltose, maltose, maltotriose, maltulose, melibiose, melezitose, palatinose, nigerose, erlose, and raffinose are also present (De La Fuente et al., 2011). The composition of honey sugars varies with factors like botanical origin, geographical location, climate, processing method, and storage conditions (Escuredo et al., 2014). The fructose-glucose ratio can differentiate between monofloral honeys, and honeydew honeys contain more di- and trisaccharides than blossom honey (Gleiter et al., 2006). The maltose-isomaltose ratio also varies among different types of honey (Gleiter et al., 2006). The sugar composition can change during storage due to enzymatic activity and acid reversion. According to Codex Alimentarius standards, the combined fructose and glucose content in honey should not be less than 60g/100g, except for honeydew and blends of honeydew with blossom honey, which should not be less than 45g/100g

(Codex Standard for Honey, 1981).

2.1.5.3. Protein & amino acids profile

Proteins were discovered in honey through color testing in 1911 (Moreau, 1911). The protein content in honey ranges from 0.1-3.3% in *Apis cerana* honey and 0.2-1.6% in Apis mellifera honey due to enzymes introduced by bees and derived from nectar (Won et al., 2009). Honey contains 26 amino acids, with proline being the dominant component and varying concentrations depending on origin (Hermosín et al., 2003). Pollen, as the primary source of honey amino acids, determines the amino acid profile and provides information about its botanical origin (Hermosín et al., 2003).

2.1.5.4. Organic acids

Organic acids in honey are essential in determining its color, taste, pH, and antibacterial and antioxidant properties (da Silva et al., 2016). They also enable the discrimination of honey's botanical origin. Honey contains a mixture of acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic, and succinic acids, which contribute to approximately 0.57% of its composition (da Silva et al., 2016).

2.1.5.5. Vitamins

Honey is a supersaturated sugar solution with low-fat content, making most of its vitamins water-soluble (da Silva et al., 2016). Honey contains trace levels of vitamin B complex derived from pollen grains, including thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8), and folic acid (B9) (da Silva et al., 2016). Vitamin C, also found in all types of honey, is well-known for its antioxidant properties but is unstable and susceptible to chemical and enzymatic oxidation (León-Ruiz et al., 2013; da Silva et al., 2016). Due to the low pH of honey, its vitamins are well-preserved (Bonté & Desmoulière, 2013; da Silva et al., 2016).

2.1.5.6. Minerals

Honey is rich in minerals, including macrominerals like potassium, calcium, and sodium, and trace minerals like iron, copper, zinc, and manganese (Alqarni et al., 2014; Rodríguez-Ramos et al., 2020). Potassium is the most abundant element, accounting for about one-third of the total mineral content, which can range from 0.02 g/100 g to 1.03 g/100 g (Alqarni et al., 2014; Bogdanov et al., 2007; Chakir et al., 2011; White Jr, 1957). Mineral content varies based on the geographical and botanical origin of the honey, with darker honeys typically containing more minerals than lighter ones (da Silva et al., 2016). Minerals in honey are indestructible and can withstand exposure to environmental factors that affect organic nutrition (da Silva et al., 2016; Damodaran & Parkin, 2018). Honey's mineral content has been used to classify its botanical origin (Ajtony et al., 2007; da Silva et al., 2016; Nozal Nalda et al., 2005).

2.1.5.7. Volatile compounds

Honey aroma is influenced by its complex mixtures of volatile organic compounds (VOCs), which vary based on nectar, processing, origin, and storage conditions (Castro-Vázquez et al., 2007). Over 600 VOCs have been identified in honey, contributing to different floral types (da Costa et al., 2018; Patrignani et al., 2018). The chemical groups in the volatile compounds in honey include hydrocarbon, aldehyde, alcohol, ketone, acid, ester, benzene, furan, pyran, norisoprenoids, terpenes, and cyclic compounds (Gianelli Barra et al., 2010). Honey aroma compounds, such as methyl anthranilate for citrus honey (Alissandrakis et al., 2007), pentanal, furfural, and 2-ethyl hexanol for buckwheat honey (Wardencki et al., 2009), and 3-caren-2-ol, p-cymene, and its derivative alcohol for eucalyptus honey (Castro-Vázquez et al., 2009), are useful markers for identifying the botanical or geographical origin of honey.

2.1.5.8. Phenolic profile

Honey is a rich source of antioxidants, especially polyphenols such as flavonoids and phenolic acids (Hossen et al., 2017). These compounds are responsible for the color, flavor, and functional properties of honey, and their profiles vary depending on the floral source (Hossen et al., 2017). The polyphenol profile of honey is a valuable tool for honey classification and authentication (Cianciosi et al., 2018; Halagarda et al., 2020), with specific compounds acting as markers for different floral types, such as hesperetin for citrus honey and kaempferol for rosemary honey (Pyrzynska & Biesaga, 2009). Honey contains two major groups of polyphenols, flavonoids and phenolic acids (Hossen et al., 2017). On average, honey contains approximately 20 mg/kg of flavonoids, which are the predominant polyphenols found in this natural sweetener (Gil et al., 1995), and are classified based on their oxidation level, with flavanones, flavanols, and flavonols being the most commonly found types (Cianciosi et al., 2018).

2.1.6. Honey production & processing

The honey production process is a complex one that is influenced by various factors. According to McHugh (2017), a colony of bees can produce an average of 80 pounds of surplus honey per year (McHugh, 2017). Foraging bees collect nectar, the main ingredient in honey, which provides carbohydrates for the colony (Gojmerac, 1980). Various factors such as weather, distance, food quality, and nectar/pollen availability influence foraging behavior (Gojmerac, 1980; Ministry of Agriculture, 2022). Upon returning to the hive, foraging bees deposit nectar to house bees and add saliva containing invertase to convert complex carbohydrates to simple sugars (Ministry of Agriculture, 2022). The partially mature honey is deposited in comb cells during heavy nectar flow, while during moderate or weak flow, the food is transferred among many bees before storage

(Ministry of Agriculture, 2022). The ripening process starts with the bee pumping out nectar into a flat drop on its proboscis, exposing it to the heated air in the hive for evaporation (Crane, 1980). The ultimate ripening takes 1 to 3 days and depends on factors such as initial water content, cell filling level, air movement, temperature, and humidity (Ministry of Agriculture, 2022).

Honey collection involves several steps, including pacifying bees with smoke, uncapping the honeycomb, and extracting honey from the cells (McHugh, 2017). Raw honey has a thick consistency and is often heated to reduce viscosity, slow crystallization, and kill yeast cells, extending its shelf-life (McHugh, 2017; Baglio, 2018). Filtering honey is essential for delaying crystallization and producing specific honey varieties. Different filtering techniques result in different honey varieties, and the choice of after-extraction heating process depends on the amount of production and desired final product. Membrane filters, macro-filtration, microfiltration, ultrafiltration, and diatomaceous earth are common filtering techniques (Subramanian et al., 2007).

2.1.7. Physicochemical properties of honey

Several commonly measured physicochemical parameters, including moisture content, water activity, pH, free acidity, color, HMF, electrical conductivity, and diastase activity, are closely related to the quality and identity of honey(da Silva et al., 2016). Honey has unique characteristics that vary widely among different types, such as buckwheat honey's dark color and strong odor and sweet clover honey's light color and mild aroma and flavor (Root, 1959). In Canada, the Canadian Food Inspection Agency (CFIA) has established specific standards for water content and other characteristics that honey must meet (CFIA, 2021). In the next section, we will explore in greater detail the various physicochemical properties of honey.

2.1.7.1. Moisture and water activity

Water content is an important property of honey as it affects crystallization, viscosity, flavor, color, taste, specific gravity, preservation, and solubility(da Silva et al., 2016). The moisture content in honey is influenced by factors such as botanical source, climate, degree of maturity in the hive, storage conditions, and processing techniques (Karabagias et al., 2014; The Codex Alimentarius, 2019; Yücel & Sultanog, 2013. Blossom honey generally has higher water content than honeydew honey, and heather honey is known for its high-water content (da Silva et al., 2016). The average moisture content of honey is between 15-21%, and it should not exceed 20% according to the Codex Alimentarius (The Codex Alimentarius, 2019) . Water activity is more important than water content for controlling microbial growth (Chirife et al., 2006). Honey's water activity ranges between 0.5-0.65, and beyond 0.60, microbial stability may be compromised, leading to fermentation and altering honey's quality (da Silva et al., 2016).

2.1.7.2. Free acidity and pH

Honey is viscous and acidic, with a pH ranging from 3.2 to 4.5 (Solayman Md et al., 2016). Although there is no established pH limit, honey's acidity contributes to inhibiting the growth of microorganisms as their optimal pH is between 7.2 and 7.4 (Karabagias et al., 2014).

The free acidity of honey, which refers to organic acids in equilibrium with lactone, inorganic ions such as phosphates, sulfates, chlorides, and internal esters (da Silva et al., 2016), is also a crucial parameter related to honey's quality. The Codex Alimentarius Committee on sugars limits free acidity to 50.00 meq kg^{-1} (da Silva et al., 2016; The Codex Alimentarius). Higher levels may indicate sugar fermentation into organic acids. However, the acidity of honey can vary due to different organic acids, geographical origin, and harvest season (da Silva et al., 2016).

2.1.7.3. Color

Honey color is critical in its commercialization as it impacts consumer acceptance and preference (Tuberoso et al., 2014). Honey can range from clear and light yellow to dark amber or nearly black, with different colors commanding different prices in different regions (Tuberoso et al., 2014). The Canadian Food Inspection Agency has two categories for honey color, each with various classes that are assigned using a honey classifier or Pfund honey grader (Canadian Food Inspection Agency, 2021). Honey color is also influenced by its botanical origin, and color analysis being crucial for identifying single-source honeys (Siddiqui et al., 2017).

2.1.7.4. 5-hydroxymethylfurfural (5-HMF)

Heating honey during processing can decrease its viscosity or melt crystallized honey, but it can also cause the loss of thermolabile aromatic compounds proportionally to the temperature and heating time (Tosi et al., 2008). Quality control parameters such as 5-HMF and diastase activity can be used to assess damages caused by heating, and high levels of 5-HMF can indicate inadequate storage and overheating conditions (Tosi et al., 2008). However, other factors, such as the sugar profile, pH, and moisture content, can influence 5-HMF levels. The Codex Alimentarius Committee stipulates a maximum limit of 40.00 $mg.kg^{-1}$ for HMF in processed honey and a maximum value of 80.00 $mg.kg^{-1}$ for honey and honey blends with a declared provenance from tropical climate (The Codex Alimentarius, 2019).

2.1.7.5. Diastase Activity

Diastase activity is used as a quality indicator for honey freshness, as it decreases over time along with 5-HMF and can be lowered by overheating during processing (Ahmed et al., 2013; da Silva et al., 2016; Yücel & Sultanoğlu, 2013). The diastase number is expressed in Schade units, and

the Codex Alimentarius stipulates a minimum value of 8.00 Schade units with a minimum of 3 Schade units for naturally lower activity in honey containing up to 15 $mg.kg^{-1}$ of HMF (The Codex Alimentarius, 2019). Diastase content can be influenced by various factors such as nectar collection period, bee age, and nectar quantity and sugar content, leading to variations in the enzyme and pollen content (Oddo et al., 1999).

2.1.7.6. Electrical Conductivity

Electrical conductivity (EC) measures a material's ability to carry an electrical current (Yücel & Sultanog˘lu, 2013) and is used to differentiate between floral and honeydew honey, with honeydew having higher levels of EC due to its mineral content (Kıvrak et al., 2017). This parameter is measured with a conductometer and has replaced ash content as the primary method for identifying honey origin (El Sohaimy et al., 2015). European regulations stipulate that honeydew and chest honey must have an EC higher than 0.8 mS/cm, while blossom honey must have an EC lower than 0.8 mS/cm (European Council, 2002). According to the Codex Alimentarius, the maximum acceptable EC value is set at 0.8 mS/cm (The Codex Alimentarius, 2019).

2.1.7.7. Key knowledge gaps

Despite the growing popularity of urban beekeeping, there remains a significant knowledge gap when it comes to understanding the physicochemical properties of urban honey. While there is general information available on the physicochemical properties of city honey (Matović et al., 2018; Preti & Tarola, 2021), there is a lack of specific data pertaining to urban honey. Detailed research is needed to fill this gap and gain a comprehensive understanding of the unique physicochemical properties of honey produced in urban environments. In future studies,

researchers could investigate factors such as the impact of urban pollution, foraging patterns of urban bees, and the influence of urban vegetation on the physicochemical properties of urban honey, to further expand our knowledge in this area.

2.2. Contaminants in urban honey

2.2.1. Heavy metals

Heavy metal pollution is a major concern in densely populated areas, particularly those with high levels of industrial activity (Bilandžić et al., 2011). Many studies have found a correlation between heavy metal levels in honey and industrial pollution in the region where the honey is harvested (Bilandžić et al., 2011; Smith et al., 2021). For example, the city of Baia Mare and its surroundings are among the most contaminated locations with heavy metals in Romania and the world, with Cu, Zn, Cd, and Pb being the primary industrial pollutants. Berinde and Michnea (2013) analyzed honey samples collected from that area. They found a positive relationship between metal concentrations in honey and those in air, settling dust and soils from the same area.

2.2.2. Air pollutants

Anthropogenic activities release contaminants that exceed the environment's ability to self-purify, leading to severe air pollution and health problems like respiratory problems and lung cancer (Cunningham, 2022; Dockery, 1993). Persistent organic pollutants (POPs) can travel long distances and resist degradation (Cunningham, 2022). Bees encounter different air pollutants during their search for food, with polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) being the most commonly found (Toptanci, 2022). Toptanci (2022) conducted a recent study on 50 urban honey samples from Turkey, measuring the levels of 16 PAHs with

QuEChERS and GC/MS/MS. The study discovered higher PAH concentrations in urban honey than rural honey, with 68% of the samples containing naphthalene, 20% acenaphthylene, and 17% acenaphthene. Dobrinas (2008) in Romania and Ozoani (2020) in Nigeria also reported similar findings.

2.2.3. Veterinary drugs

Honeybees are often exposed to antibiotics and acaricides used by beekeepers to protect their hives against diseases such as European foulbrood, American foulbrood, and nosemosis, which can end up in honey and lead to contamination (von Eyken et al., 2019). Antibiotics such as tetracycline, tylosin, and sulfonamides are commonly used for bacterial infections prevention or treatment (Reybroeck et al., 2012; Annie von Eyken et al., 2019), while acaricides like fluvalinate, flumethrin, pyrethroids, amitraz, and coumaphos are used to treat bee mites and sometimes reported as pesticides in food safety reports (Sammataro, 2012). It is important to note that various veterinary drugs are often reported as pesticides, too, as they fit into both categories. To date, no studies have investigated the levels of veterinary drugs in urban honey or if different regions impact their use (Gonçalves Lima et al., 2020; Kivrak et al., 2016; Korkmaz et al., 2017).

2.2.4. Pesticides

Pesticides are a group of chemicals that are commonly used in agriculture, forestry, and urban areas to control pests, weeds, and diseases (Sharma et al., 2019). They are essential in modern agriculture, protecting crops from pests and diseases (Sharma et al., 2019). In urban areas, pesticides are used to control pests like mosquitoes, cockroaches, and rats and also to regulate the growth of undesired species of grasses (Md Meftaul et al., 2020). However, the widespread use of

pesticides to control bee diseases and pests is often done without proper regulation and oversight, which can have harmful effects on bees and other pollinators. Moreover, pesticides can contaminate the pollen and nectar that bees collect and have been associated with the death of entire colonies. This can negatively impact crop yields and food security as pollinators play an important role in crop production. In the following sections, we will discuss pesticides further and the issue of pesticides in urban honey.

2.2.4.1. Classification of pesticides

Pesticides encompass various chemicals, including insecticides, fungicides, herbicides, rodenticides, wood preservatives, garden chemicals, and household disinfectants (Eldridge, 2008; Yadav & Devi, 2017). These pesticides exhibit diverse physical and chemical properties making it beneficial to classify them based on their characteristics and study them within their respective groups. Synthetic pesticides are chemically engineered compounds that are not naturally occurring and can be categorized into various types based on their intended use. Drum proposed three popular methods of pesticide classification (Drum, 1980), which include: (i) classification based on the mode of entry, (ii) classification based on the function of the pesticide and the pest it targets, and (iii) classification based on the chemical composition of the pesticide (Drum, 1980).

Pesticides can reach their intended target through various entry modes, such as systemic absorption, direct contact, ingestion, fumigation, and repellency (Yadav & Devi, 2017). Systemic pesticides, like glyphosate and 2,4-D, can be taken in by plants or animals and spread to other untreated areas (Ed & Transl, 1983). In contrast, non-systemic or contact pesticides, such as paraquat and diquat dibromide, only work when they come into direct contact with the target pests (Yadav & Devi, 2017). Stomach poisoning pesticides like malathion are ingested by pests and

cause death through the digestive system (Government of Newfoundland and Labrador, 2005); (Yadav & Devi, 2017). Fumigants release poisonous gases to kill pests (Yadav & Devi, 2017), while repellents make treated areas or commodities unappealing to pests, interfering with their ability to locate crops (Yadav & Devi, 2017).

Under this classification system, pesticides are classified and named based on their target pest organism and mode of action, using the Latin term "*cide*" as a suffix to reflect their killing action (Yadav & Devi, 2017). For instance, inescticides are used to kill insects. Not all pesticides are named with the suffix "-cide". Some pesticides are categorized based on their purpose, such as growth regulators, which can either stimulate or inhibit the growth of pests (Yadav & Devi, 2017).

Pesticides can be categorized based on their chemical composition and active ingredients, offering important information about their effectiveness and physical properties (Yadav & Devi, 2017). This knowledge is crucial for determining the best application methods, safety precautions, and appropriate dosage rates. For example, glyphosate is a widely used herbicide with a phosphonic acid-based active ingredient, while pyrethrins are natural insecticides derived from chrysanthemum flowers.

2.2.4.2. Pesticides in the urban environment

The urban environment is characterized by high population density and human infrastructure, such as buildings, roads, and green spaces, that has developed as a result of urbanization (Galea et al., 2007; Md Meftaul et al., 2020; Pickett et al., 2011). Proper management of urban areas is crucial for ecological, economic, and social sustainability (Pickett et al., 2011; Platt et al., 1994). However, pests threaten these areas, and pesticides are commonly used to manage them (Md

Meftaul et al., 2020). Urban pest control programs use a wide range of pesticides, including insecticides, herbicides, fungicides, rodenticides, miticides, repellents, and fumigants (WHO, 2006). Pesticides can be released into the urban environment through various means, including production, transportation, and improper storage or usage, as well as proper usage, leading to significant environmental problems (Md Meftaul et al., 2020; Relyea, 2005). Soil can act as a receptacle for pesticides that enter the environment via various methods, such as direct application, sewage sludge application, and atmospheric deposition (Md Meftaul et al., 2020; Wang et al., 2015). Wastewater treatment plants (WWTPs) and the use of sewage sludge as a soil amendment are also significant sources of pesticide pollution in urban areas (Md Meftaul et al., 2020). Organic contaminants can easily enter the environment through the use of biosolids, which are often used in agriculture and urban areas (Clarke & Smith, 2011; Md Meftaul et al., 2020). Various pesticides can persist in the environment for long periods and can be absorbed by plants, including those used by bees to produce honey. As a result, urban honey can contain residues of pesticides, which can be harmful to human health if consumed in large quantities.

2.2.4.3. Pesticides in urban honey

Several studies have shown that pesticides are regularly detected in food products, leading to significant exposure to these harmful substances through our diet (Md Meftaul et al., 2020; Prodhan et al., 2018). Some pesticides, even in small amounts, can negatively impact our health by causing harm to our brain and reproductive systems and potentially causing cancer. Contamination of honey can occur directly (for instance, honey bee colonies subjected to treatments for veterinary reasons) or indirectly through the environment where bees collect nectar, emphasizing the need to regulate pesticide use in honey production to safeguard food safety and the environment (Blasco et al., 2011; Souza Tette et al., 2016). Relevant literature pertaining to

the subject of interest was sought by consulting databases including ScienceDirect, Web of Science, PubMed, and Google Scholar restricting the search to the most recent five years (2016-2021). **Table 2** ***1*** summarizes the commonly reported pesticides, along with their concentration ranges in honey, from 2016 to 2021 across 29 studies.

	Compound	**CAS number**	**Chemical formula**	**Molecular weight**	**Honey (ng/g)**	**References**
1	Coumaphos	14-72	$C_{14}H_{16}ClO_5PS$	362.77	1.55-23	(Bommuraj et al., 2019; Chiesa et al., 2016; Gawel et al., 2019; Juan-Borrás et al., 2016; Laaniste et al., 2016; Saitta et al., 2017; Valdovinos-Flores et al., 2016)
2	Clothianidin	210880-92-5	$C_6H_8ClN_5O_2S$	249.68	0.32-63	(Jones & Turnbull, 2016; Kavanagh et al., 2021; Mitchell et al., 2017; Rolke et al., 2016; Song et al., 2018; Woodcock et al., 2018)
3	Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.01-50	(Bommuraj et al., 2019; Jiang et al., 2018; Jones & Turnbull, 2016; Song et al., 2018; Valdovinos-Flores et al., 2017; Woodcock et al., 2018)
4	Thiamethoxam	153719-23-4	$C_8H_{10}ClN_5O_3S$	291.72	0.29-65.5	(Codling et al., 2016; Jiang et al., 2018; Mitchell et al., 2017; Song et al., 2018; Woodcock et al., 2018)
5	Thiacloprid	111988-49-9	$C_{10}H_9ClN_4S$	252.72	1.4-130	(Baša Česnik et al., 2019; Bommuraj et al., 2019; Gawel et al., 2019; Karise et al., 2017; Laaniste et al., 2016; Mitchell et al., 2017; Song et al., 2018)
6	Tau-fluvalinate	102851-06-9	$C_{26}H_{22}ClF_3N_2O_3$	502.9	2-10	(Gawel et al., 2019; Juan-Borrás et al.,

						2016; Karise et al., 2017; Shendy et al., 2016)
7	Amitraz	33089-61-1	$C_{19}H_{23}N_3$	293.4	1-177	(Baša Česnik et al., 2019; Gawel et al., 2019; Juan-Borrás et al., 2016; Juan-Borrás et al., 2014; Lozano et al., 2019)
8	Glyphosate	1071-83-6	$C_3H_8NO_5P$	169.07	3-118	(Berg et al., 2018; Karise et al., 2017; Thompson et al., 2019; Zoller et al., 2018)
9	Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	350.6	0.52-133.4	(Chiesa et al., 2016; Darko et al., 2017; Deng et al., 2017; Irungu et al., 2016; Kumar et al., 2018; Lozano et al., 2019; Piechowicz et al., 2018; Rafique et al., 2018; Saitta et al., 2017)
10	Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.101	1.13-17.2	(Chiesa et al., 2016; Darko et al., 2017; Gawel et al., 2019; Irungu et al., 2016; Saitta et al., 2017)

Table 2 1 Summary of some commonly reported pesticides in honey from 2016 -2021 in different countries.

The available data and scientific studies on pesticide analysis in urban honey are scarce. Neonicotinoids (NIs), which are among the most in-demand and widely employed pesticides at present, have been utilized extensively. These insecticides, used to protect crops, pets, and trees, include imidacloprid, thiamethoxam, thiacloprid, acetamiprid, nitenpyram, clothianidin, and dinotefuran (Cicero et al., 2017). They are widely used on key crops in Canada, such as canola, wheat, barley, oats, and field peas (Codling et al., 2016). These and other pesticides have been linked to colony collapse disorder and other harmful effects on honeybees, such as reduced

immunity and memory impairment (DesJardins et al., 2021; Tosi et al., 2021; Tsvetkov et al., 2017). In response, Canada has banned the use of NIs on attractive crops for bees and banned spraying of certain crops during bloom (Government of Canada, 2020). Currently, imidacloprid, thiamethoxam, and clothianidin are the only neonicotinoids approved for agriculture in Canada (Government of Canada, 2020). Although NI levels are not usually detected by Canadian Food Inspection Agency tests (CFIA, 2015-2016), levels reported globally range from 1-10 $ng.g^{-1}$ in honey (Codling et al., 2018).

Numerous studies have documented the widespread presence of NIs in honey samples across the globe (Kavanagh et al., 2021; Lambert et al., 2013; Mullin et al., 2010). Mitchell et al. (2017) found that at least one of five tested NIs was detected in 75% of 198 honey samples from all continents, with 45% containing two or more of these compounds and 10% containing 4 or 5 of these compounds (Mitchell et al., 2017). A 2019 study in Poland reported that the most frequently detected NIs in 77% of 155 honey samples were acetamiprid and thiacloprid, while fungicides and amitraz were found in 50 and 30% of samples, respectively (Gawel et al., 2019). In Canada, a 2016 study analyzed 26 honey samples from Saskatchewan and discovered clothianidin and thiamethoxam as the most prevalent NIs, present in 68 and 75% of samples, respectively, with mean concentrations of 8.2 and 17.2 $ng.g^{-1}$ wet weight which is below the Canadian maximum residue limit (MRL) of 0.1 $mg.kg^{-1}$ (Codling et al., 2016).

Glyphosate, a widely used herbicide in Canada, is an essential tool for weed management in both agricultural and non-agricultural lands. In terms of human health, glyphosate has been associated with a wide range of disorders and diseases, including Alzheimer's, autism, cancers, and more

(Panseri et al., 2020). This herbicide has been found in honey samples from countries worldwide (**Table 2** ***2***), such as Switzerland and various US states (Rubio et al., 2014; Zoller et al., 2018). A study in Canada found that nearly all honey samples (n=200) tested contained residues of glyphosate exceeding the limit of quantification (LOQ) of 1 $ng.g^{-1}$ (98.5%) (Thompson et al., 2019). In Hawaii, high concentrations of glyphosate residues were identified in hives near agricultural lands, extensive golf courses, and highways (Berg et al., 2018). These results imply that honey produced in urban areas situated near these glyphosate-treated environments may be subjected to contamination, especially considering the numerous golf courses present in urban settings. While there is no maximum residue limit (MRL) for glyphosate in the US or Canada, in Europe, the MRL for glyphosate is set at 0.05 $mg.kg^{-1}$ in honey(Kerri-Jane McAlinden, 2021).

Despite a plethora of research on pesticide residues in honey produced in agricultural fields, there is a lack of information on pesticide residues in honey produced in cities. The presence of pesticides in food has become a global concern for consumer safety, and people have the right to be informed about the levels of pesticide residue in their food (Hasan et al., 2017). Studies that have assessed the presence of pesticides in urban honey have been limited, and the results have been inconsistent. For example, Jovetic and colleagues (2018) have assessed urban honey and bee pollen samples for pesticide residues using gas chromatography coupled to mass spectrometry (GC-MS). Both matrices showed one or more of the 123 pesticides examined. None of the detected pesticide residues were above the LOQ of the method, 0.01 mg kg^{-1} (Jovetic et al., 2018). In 2013, Lambert et al., (2013) stated slightly higher contamination of rural honey than honey from other sites, including urban (Lambert et al., 2013). In a recent study by Kavanagh et al., urban honey was analyzed using ultra-high performance liquid chromatography- mass spectrometry (UHPLC-

MS) and clothianidin and thiacloprid were more commonly detected in honey from urban habitats, demonstrating that pesticide exposure does not solely occur in rural regions (Kavanagh et al., 2021).

2.3. Analysis of pesticides in honey

Analyzing pesticide residues in food, particularly honey has been an ongoing research area in recent years. Different analytical techniques have been used to detect and quantify these residues in honey samples (**Table 2** ***2***), with liquid chromatography (LC) and GC being the most widely used (Souza Tette et al., 2016). The choice of separation process is largely determined by the properties of the pesticides of interest. GC can detect volatile, semi-volatile, and thermally stable chemicals, whereas LC can determine non-volatile and/or thermally unstable molecules (Kujawski et al., 2014). GC-MS was traditionally the most commonly used technology for analyzing residues in foods (Farré et al., 2014), but as pesticides have become more polar, thermally unstable, or difficult to evaporate, LC-MS has become more prevalent (Souza Tette et al., 2016). LC-MS is increasingly popular for detecting, identifying, and quantifying pesticides in food due to its wide range of coverage and ease of sample preparation (Stachniuk & Fornal, 2016). This method provides structural information about the analyte without the need for derivatization, and it has relatively lenient sample purity requirements (Stachniuk & Fornal, 2016). Additionally, LC-MS allows for the simultaneous analysis of substances with a wide range of polarities (Stachniuk & Fornal, 2016). The trend is further supported by the increasing number of studies and publications dedicated to the application of LC-MS in the determination of contaminants in food (Galani et al., 2019; Gómez-Pérez et al., 2015; Han et al., 2016; Huang et al., 2019; Rafique et al., 2018; Sivaperumal et al., 2015; Souza et al., 2021; Weng et al., 2020).

Country of study	**USA/Canada**	**USA**	**Switzerland**	**USA (Hawaii)**	**Canada**
Method	[a]ELISA	[b]LC-MS/MS	LC-MS/MS	ELISA	LC-MS/MS
Source of honey	Many countries of origin	Mostly from the USA	N/A	Hawaii	Western, Canada
Number of samples	69	28	16	85	200
Positive samples (%)	59.4	60.7	93.8	28.2	98.5
Log ($ug.kg^{-1)}$	15	10-16	1	15	1
Maximum ($ug.kg^{-1}$)	163	653	15.9	342	49.8
References	(Rubio et al., 2014)	(Chamkasem & Vargo, 2017)	(Zoller et al., 2018)	(Berg et al., 2018)	(Thompson et al., 2019)

Table 2 2 Glyphosate in honey from worldwide countries

[a]ELISA = enzyme-linked immunosorbent assay, [b]LC-MS/MS = liquid chromatography-tandem mass spectrometry

Conventionally, pesticide residues in honey are analyzed using a targeted approach which requires an extraction and clean-up process prior to quantification using LC-MS or LC-MS/MS. Commonly used sample preparation methods, such as liquid-liquid extraction (LLE), have significant drawbacks in terms of cost, time consumption, and the use of large volumes of toxic organic solvents that pose a risk to the safety of technicians and the environment(Souza Tette et al., 2016).

Moreover, traditional purification methods may remove additional compounds important for the present or future evaluation of honey samples, such as the presence of additional pollutants, chemical markers, metabolites, etc. (A. von Eyken et al., 2019). Instead of using complex sample preparation methods, a new approach called direct injection or dilute and shoot has been developed to rapidly analyze trace compounds in food and environmental samples (A. von Eyken et al., 2019). Generally, samples are diluted using a mixture of acetonitrile and water and then directly loaded into an LC-MS. This method is usually used for matrices with lox complexity and high levels of analytes, such as honey (Gómez-Pérez et al., 2012; Mol et al., 2008; A. von Eyken et al., 2019).

Multi-targeted LC-MS/MS methods have been widely used for this purpose, allowing for the simultaneous analysis of a large number of compounds. However, these methods are limited to a predefined set of targeted pesticides and may miss other contaminants present in the sample (Bauer et al., 2018). Moreover, the applicability of these targeted methods is hindered by a lack of available standard references, particularly for pesticide transformation products (Bauer et al., 2018), making it challenging to fully map the pesticide profile in food samples (Guo et al., 2020). As a result, non-targeted analysis (NTA) has gained popularity as a promising approach for monitoring pesticide residues in food samples, as it has been extensively studied in various food matrices (Hayward et al., 2011; Kunzelmann et al., 2018; Picó et al., 2018; Prata et al., 2022) and has shown effectiveness. However, research in the analysis of pesticides in honey using NTA is still limited, and only a few studies have been conducted in this area. One notable study by von Eyken and Bayen in 2019 utilized a QTOF analyzer to identify potential contaminants in honey samples from the Canadian market using a suspect list of 43 compounds, identifying four pesticides, two veterinary drugs, and two other contaminants through MS/MS matching analysis

(von Eyken & Bayen, 2019). Similarly, Gómez-Pérez et al. (2015) developed a similar approach using Exactive Orbitrap MS to retrospectively screen pesticides and veterinary drugs' transformation products (Gómez-Pérez et al., 2015).

In conclusion, the need for non-targeted analysis studies on pesticides in urban honey cannot be overstated. With the increased urbanization and expansion of beekeeping in cities, the potential exposure of honeybees to environmental contaminants has become a major concern. The limitations of targeted analysis methods have highlighted the need for NTA approaches, which have shown effectiveness in various food matrices. However, research in this area for honey is still limited, and there is a need for more studies utilizing NTA methods to identify and monitor the presence of pesticides in urban honey. The development of such methods will aid in ensuring food safety and promoting sustainable urban beekeeping practices.

2.4. Monitoring of airborne pesticides in urban areas

The extensive utilization of pesticides has resulted in pollution of all areas of the ecosystem. The atmosphere is recognized as an effective means for the global distribution of pesticides (Glotfelty et al., 1989; Tadeo & L, 2019). Pesticides have the ability to access the atmosphere through several means, such as spray drift during the application, volatilization from soils and leaves after application, and wind erosion, which can carry pesticides attached to soil particles and transport them into the atmosphere (Glotfelty et al., 1989; Tadeo & L, 2019). During pesticide spray, about 70% of pesticides reach their targets, while the rest remains in the air (Fuhrimann et al., 2020; Van Den Berg et al., 1999). When present in the atmosphere, pesticides may be apportioned between the gaseous and particulate phases, and this partitioning is dependent on the physical and chemical

characteristics of the pesticide as well as various environmental and climatic factors, such as temperature and humidity (Tadeo & L, 2019).

Studies have revealed that pesticides can be transported far from their application sites, depending on their persistence (Degrendele et al., 2016; Zhang, Meyer, et al., 2013). Airborne pesticides can be dispersed and transported to urban and remote regions, making the atmosphere an ideal medium for their distribution (Shen et al., 2005). Studies conducted in Europe (Schummer et al., 2012), Africa (Fuhrimann et al., 2020; Isogai et al., 2018), Asia (Shunthirasingham et al., 2010) , North America (Daly et al., 2007), and even at the North and South Poles (Bengtson Nash et al., 2017) have shown that pesticides are present in the air all over the world (Martin et al., 2022). These findings stress the importance of addressing the distribution of pesticides via the atmosphere to reduce the potential harm to the environment.

2.4.1. Methods for detecting and measuring airborne pesticides

Air sampling techniques are essential for detecting low concentrations of pesticides in ambient air (Yusà et al., 2009). The most commonly used method is air pumping onto traps, which is limited in its effectiveness due to the low concentrations of pesticides present in the air (Tadeo & L, 2019). Two main techniques, passive air sampling (PAS) and active air sampling (AAS), are used for an accurate qualitative and quantitative assessment of air quality(Wang et al., 2016). While AAS has been the traditional method for sampling pesticides for many years (Al-Alam et al., 2021; Lévy et al., 2020; Tuduri et al., 2012), PAS allows for integrated measurement of pesticide concentrations over a longer sampling period and is often favored in environmental studies due to its cost-effectiveness(Gamboa et al., 2020).

Passive air sampling works by allowing chemicals in the air to diffuse onto a sampling material, such as polyurethane foam (PUF) (Harner et al., 2004; Shoeib & Harner, 2002), sorbent impregnated PUF(Genualdi et al., 2010), or XAD resin (Zhang et al., 2017; Zhang, Brown, et al., 2013). XAD resin-based passive air samplers (XAD-PAS) have a high uptake capacity of semi-volatile organic compounds (SVOCs) and can be deployed for several months to years (Wania et al., 2003). Pesticides can be distributed in both the vapor and particulate phases through a process known as vapor-particulate (V/P) partitioning (Yusà et al., 2009). Once pesticides are released into the atmosphere, they are subject to potential long-range transport and/or chemical transformations. The concentration of pesticides in the atmosphere is significantly diminished primarily through chemical degradation and deposition mechanisms (Socorro et al., 2016). Recent studies have demonstrated that the concentration of currently used pesticides in both the gas and particulate phases ranges from 0.08 to 30 ng m^{-3} (Coscollà et al., 2017; López et al., 2017).

PAS has become a popular method for detecting pesticides in the environment, and PUF disks and XAD-2 samplers are primarily used for detecting pesticides (Bogdal et al., 2013; Yusà et al., 2009). Not all pesticides are equally detectable by PAS, with some being more volatile and evaporating into the air more readily, making them easier to detect, while others are less volatile and require longer sampling times or more sensitive detection methods. PAS have been shown to be adequate for studying organochlorine, organophosphorous, pyrethroid or carbamate pesticides, as well as several other persistent organic pollutants (Nascimento et al., 2018). XAD has been used to collect various pesticides such as chlorpyrifos, fonofos, mevinphos, phorate, terbufos, diazinon, cyanazine, simazine, alachlor, atrazine, deethyl atrazine, deisopropyl atrazine, molinate, methyl parathion, hexachlorobenzene, dichlorvos, and trifluralin (Coscollà et al., 2010; Coscollà et al.,

2013; Liaud et al., 2016; Peck & Hornbuckle, 2005; Scheyer et al., 2007; Schummer et al., 2012; Tadeo & L, 2019).

2.4.2. Assessing pesticide contamination in urban areas using passive air sampling

PASs have been widely employed in urban areas for detection of pesticides (Baraud et al., 2003; Coscollà et al., 2010; Coscollà et al., 2013; Gouin et al., 2008; Schummer et al., 2010; Wang et al., 2017). In 2018, Lévy et al., monitored pesticide concentrations and variations in the ambient air at three different sites (rural, urban, and suburban) in France for 4 years using XAD-resin PASs. The results showed different patterns of pollutant accumulation between the rural and urban sites, with different proportions of pesticides observed depending on whether they were applied for domestic use or released into the environment and decomposed by various environmental and meteorological conditions. Using PUF discs, in urban, suburban, and rural background air in southern Ghana, various OCPs were observed, including DDTs, HCHs, hexachlorobenzene, pentachlorobenzene, chlordanes (α-, β-chlordane, oxychlordane, and trans-nonachlor), endrins (endrin, endrin aldehyde, and endrin ketone), heptachlor (heptachlor, heptachlor epoxide A, and heptachlor epoxide B), isodrin, endosulfans (α- and β-endosulfan and endosulfan sulphate), methoxychlor, and mirex. The concentrations of individual pesticides ranged from below detection limits to 750 pg m^{-3}(Adu-Kumi et al., 2012). In the urban air of South China, a range of additional pesticides, such as allethrin, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, dimefluthrin, permethrin, tetramethrin, and chlorpyrifos were detected. The combined concentrations of these nine pesticides varied from 150 to 3816 pg m^{-3}.

2.4.3. Analysis of atmospheric pesticides

The accelerated solvent extraction (ASE) method is a commonly used technique to extract pesticides from adsorbents and filters, which reduces the duration of the extraction process and the amount of solvent required (Tadeo & L, 2019). Depending on the analytical method utilized, a post-concentration cleanup step, such as solid phase micro-extraction (SPME), may be conducted (Tadeo & L, 2019). After extraction, pesticides are typically analyzed using GC or high-performance liquid chromatography (HPLC), with different detectors employed in each (Tadeo & L, 2019). For GC, detectors like electron captured (ECD), nitrogen-phosphorus detectors, and mass detection in the SIM mode are used to assess different types of pesticides. On the other hand, HPLC typically uses diode array detectors and fluorescence detectors for carbamates after post-column derivatization, as well as MS. LC, is predominantly utilized to evaluate polar or acidic compounds (Tadeo & L, 2019).

The extensive presence of chemicals in the environment and the limited understanding of their environmental behavior and impacts have motivated the creation and implementation of NTA (Hollender et al., 2017; Zhang et al., 2020). Most NTA studies, which focus on identifying lesser-known persistent, bioaccumulative, and toxic chemicals, have concentrated on the aquatic environment using LC coupled with HRMS (Zhang et al., 2020). In a literature survey carried out by Zhang et al., covering the period from 2008 to 2019, to investigate the current trends and advancements in NTA of organic pollutants present in the atmosphere, only a single study out of 19 representative studies utilized HPLC coupled with Orbitrap to screen pesticide metabolites in air (López et al., 2016; Zhang et al., 2020). López and colleagues (2016) performed suspect screening after the analysis to identify pesticide metabolites on a list of 240 suspected compounds

and non-target screening to identify unknown metabolites in the samples collected from the surrounding environment (López et al., 2016). The utilization of NTA coupled with LC-MS in examining pesticides in the air is a field of research presently under investigation. Despite various studies that have been carried out, our understanding suggests that no research has been carried out using NTA and LC-MS to analyze airborne pesticides, especially in cities.

2.4.4. Honey as environmental bioindicators

Bioindicator has emerged as a valuable alternative to traditional monitoring methods for evaluating environmental contamination (Xu et al., 2013). Honeybees and their products, such as honey, have been considered ideal bioindicators due to their continuous exposure to pollutants in soil, water, air, and vegetation during their foraging flights (**Figure 2** ***2***)(Girotti et al., 2020; Herrero-Latorre et al., 2017). Studies have shown that honey from urban and polluted areas tends to have higher concentrations of toxic metals compared to honey from rural areas, making honey an effective bioindicator of environmental contamination (Adugna et al., 2020; Bartha et al., 2020; Bastías et al., 2013; Lambert, Piroux, et al., 2012; Smith et al., 2019). Furthermore, in addition to being effective in monitoring metals, honey, and other bee products have been found to be valuable for monitoring air pollutants and pesticides. Research suggests that they can potentially serve as a substitute for PASs (Al-Alam et al., 2019; Kazazic et al., 2020; Lambert, Veyrand, et al., 2012; Sari et al., 2020, 2021 #305), as well as for monitoring pesticides (Al Alam et al., 2017; Chiesa et al., 2016; Malhat et al., 2015; Panseri et al., 2014).

When cost limits the simultaneous use of PAS, environmental bioindicators, such as honey, is crucial for comparing and validating their performance. Comparisons have been made between different types of samplers and biomonitoring methods, such as Ginkgo leaves and active air

samples, pine needles and Hi-Vol, PUF and Tillandsia epiphytes, XAD-PAS and lichens and conifer needles, XAD-PAS and lichens, XAD-PAS and T. bergeri epiphyte, and PUF and honeybees, with varying results depending on the type of sampler and bioindicator used (Daly et al., 2007; Klánová et al., 2009; Murakami et al., 2012; Schrlau et al., 2011; Silva-Barni et al., 2019; Wannaz et al., 2013). However, no studies to date have combined results of PAS with honey samples to monitor pesticide distribution in the air in urban areas, which is essential for protecting human health, the environment, and pollinators, as well as understanding trends in pesticide use and the effectiveness of efforts to reduce or eliminate their use.

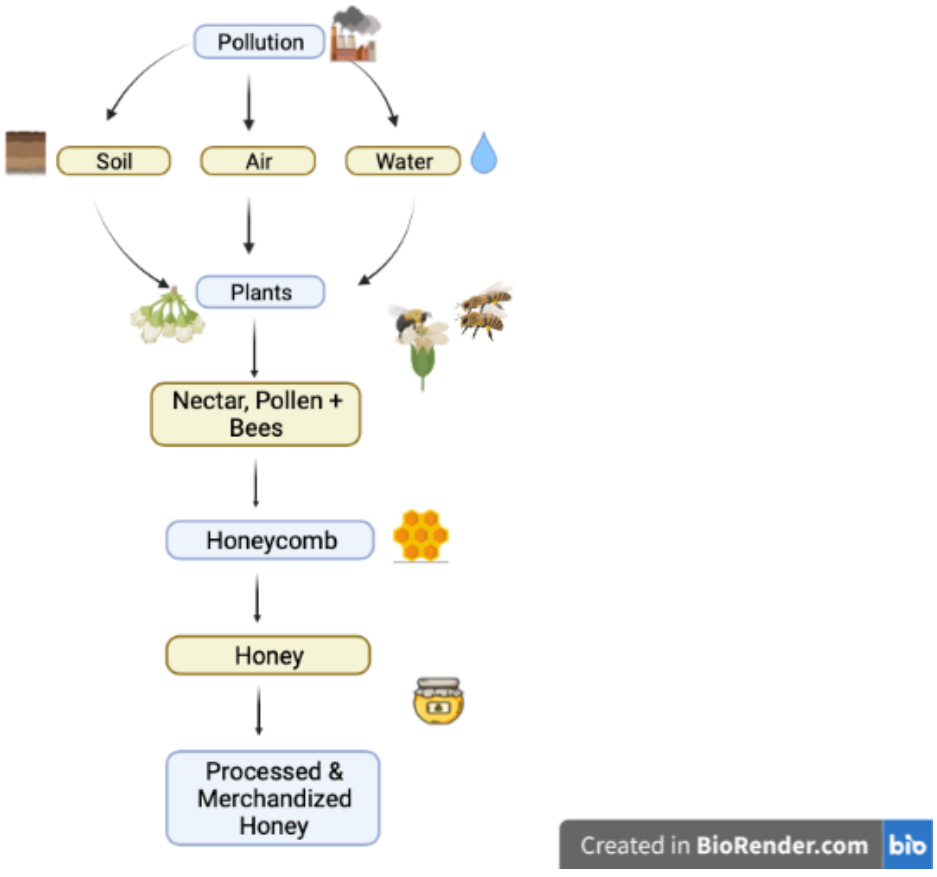

Figure 2 2 Overview of natural and anthropogenic sources of environmental pollutants in honey. Adapted from (Solayman Md et al., 2016).

2.5. Pesticide regulation and use in Canada

2.5.1. Regulations and policies related to pesticides in Canada

In Canada, pesticide regulation involves the federal, provincial, and municipal governments, which have various acts, regulations, guidelines, directives, and by-laws to mitigate the risks associated with pesticide use (CNLA, 2019). Health Canada's Pest Management Regulatory Agency (PMRA) is responsible for regulating the use, sale, manufacture, storage, and importation of pest control products in Canada under the Pest Control Products Act (PCPA) (CNLA, 2019). Each province and territory may have additional regulations that align with federal laws and may impose stricter guidelines for the use of pesticides or ban the use of certain registered pesticides (CNLA, 2019). Moreover, municipalities may establish additional regulations on the use of pesticides, including restrictions on the timing and location of use for specific types of pesticides (CNLA, 2019).

Recently, Montreal has banned the sale of 36 active ingredient and around 100 pesticides intended for domestic use, as well as the usage of specific pesticides in farming, ornamental gardening, and pest control (Ville de Montreal, 2023). Golf course operators are exempt from this ban, and professionals who use pesticides in their line of work, including farmers, exterminators, and horticulturists, will be required to obtain an annual permit to use these products (Ville de Montreal, 2023).

Health Canada evaluates the maximum residue levels of pesticides in food products prior to their registration to determine their safety for human consumption. This legal limit, called the Maximum Residue Limit (MRL), is regulated under the Pest Control Products Act (PCPA) and is established based on scientific data to ensure food safety for Canadians. The MRLs are set significantly below

any potential health risks and are applied to both raw and processed food products. Health Canada sets separate MRLs for processed food products that require higher levels than their raw agricultural commodity. In cases where a product poses an unacceptable risk, it is not allowed for sale or use in Canada. The PMRA uses a default MRL of 0.1 parts per million (ppm) until a specific MRL is determined. Health Canada establishes MRLs for honey that consider its specific characteristics, such as its composition and production, to ensure that it is safe for consumption. These MRLs include specific limits for residues of certain pesticides (**Table 2** ***3***). Different standards apply in the European Union (EU), where regulations oversee MRLs for three specific acaricides: amitraz, coumaphos, and cyamizole, with levels set at 0.2, 0.1, and 1 ppm, respectively. In the case of other pesticides, the MRL is maintained at 0.05 ppm.

Compound	**MRL (ppm)**
Amitraz	0.1
Coumaphos	0.02
Flumethrin	0.003
Tau-fluvalinate	0.02
Other pesticides	0.1

Table 2 3 MRL for pesticides in honey in Canada (Government of Canada, 2022)

2.5.2. Pesticides sale and use in Quebec

The 2020 report on pesticide sales in Quebec, published by the DMPD and MELCC, revealed that over 13 million kilograms of commercial pesticides were sold in the province (DMPD & MELCC, 2020). The majority of these sales were herbicides (58%), followed by insecticides (16%) and fungicides (11%) (DMPD & MELCC, 2020). Of the total sales, 70% were for agricultural use,

18% for urban use, and 12% for other use (**Figure 2** ***3***). The urban sales were further divided into 73% for domestic use, 16% for commercial use in the maintenance of green spaces, and 11% for professional pest management (DMPD & MELCC, 2020). The total sales of pesticides for urban use nearly doubled from 2019, potentially due to an increase in horticultural interest among the population during the pandemic. Of the urban pesticides used, 62% were biopesticides derived from natural sources, which are considered less toxic, while the remaining 38% were conventional pesticides (DMPD & MELCC, 2020). In terms of specific chemical groups, the organochlorine group was the second most sold for urban domestic use, while benzoic acids and derivatives and aryloxy carboxylic acids and derivatives were the most widely sold for urban green spaces and golf courses. For professionals in pest management, the inorganic and pyrethroid groups were the most sold, at 78% and 18% of total sales, respectively (DMPD & MELCC, 2020).

From a chemical standpoint, the organochlorine chemical group, including paradichlorobenzene, was the second most popular group of chemicals used for domestic use in urban areas (DMPD & MELCC, 2020). For urban green spaces and golf courses, the most used chemical groups were benzoic acids and derivatives, which include the herbicide dicamba, and aryloxy carboxylic acids and derivatives, which include the herbicides 2,4-D and mecoprop (DMPD & MELCC, 2020). In the pest management industry, inorganic and pyrethroid pesticides were the most popular, accounting for 78% and 18% of total sales, respectively (DMPD & MELCC, 2020).

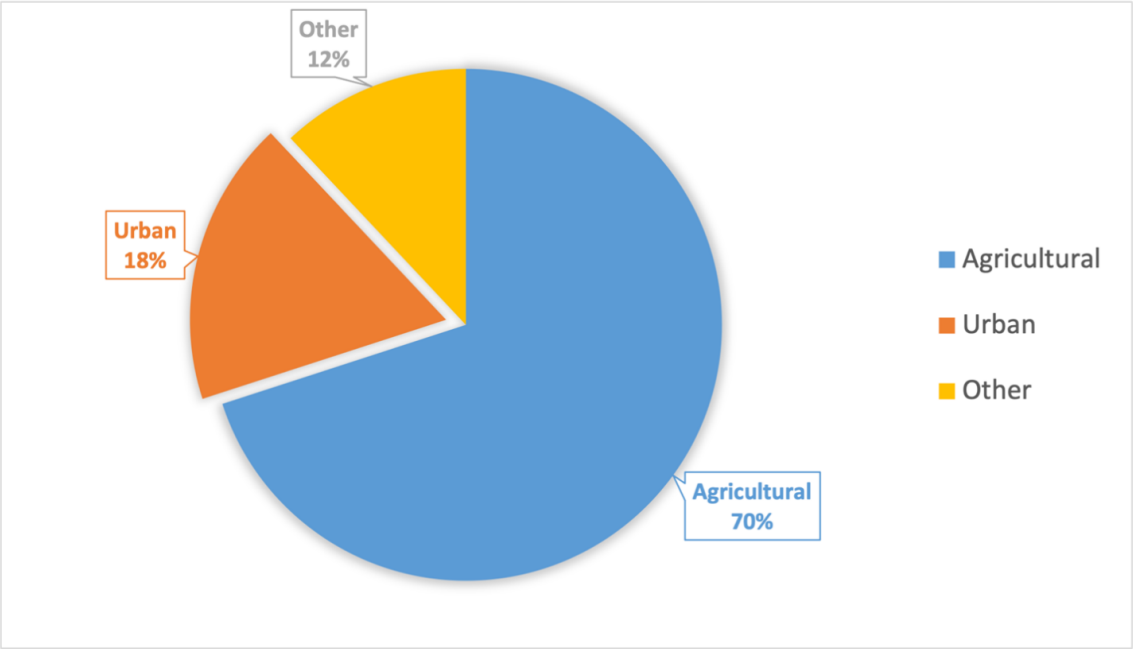

Figure 2 3 Distribution of total pesticide sales in Quebec by use settings in 2020 according to (DMPD & MELCC, 2020)

2.6. Conclusion

"The future is urban, nearly 70% of the world's population will live in cities by 2050 (UN, 2018)." As the list of contaminants in food continues to grow, new approaches to detection and identification are necessary. Non-targeted analysis techniques hold promise in providing comprehensive and reliable analysis of food contaminants, but their application to urban environments is still limited. With the increasing demand for urban apiculture and the prevalence of food contamination, assessing the safety of urban honey is vital. Recent advances in food omics and non-targeted analysis techniques offer new opportunities to screen and detect new environmental contaminants in honey. Further research is necessary to evaluate human pesticide exposure through honey consumption. Such research can provide valuable insights into environmental bioindicators for future safety assessments. Overall, additional scientific investigation is indispensable to fill the knowledge gaps and understand the potential hazards of pesticides in urban settings to safeguard public health and the environment.

CONNETING PARAGRAPH

In Chapter 2, an introduction was given on urban honey and its primary pollutants. Additionally, a brief summary was provided on the use of non-targeted analytical techniques for detecting pesticides in honey. Several gaps in knowledge were identified in this field. To fill in these gaps, Chapter 3, describes the development of a method for the simultaneous targeted and non-targeted analysis of pesticide residues in honey.

Chapter 3: Development and validation of a method for the simultaneous targeted and non-targeted screening of pesticides in urban honey

3.1. Abstract

Conventionally, the analysis of pesticide residues in honey involves intricate extraction and purification processes before quantification through high-performance liquid chromatography coupled with advanced mass spectrometry. These steps, designed to isolate target compounds, are time-consuming, costly, and potentially exclude components crucial for honey assessment. As an alternative, rapid analysis of trace compounds in environmental and food matrices has been facilitated through direct injection, eliminating the need for complex sample preparation. In this context, this study aimed to utilize a direct injection technique coupled with high-performance liquid chromatography and quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF-MS) as a non-targeted screening method for detecting pesticides in urban honey. Following SANCO guidelines, method validation was conducted with 21 pesticides as targeted compounds. The technique was validated according to the SANCO guideline recommendations for 21 key pesticides, and was assessed to be robust and sensitive for this application. It was able to detect pesticide residues 2 to 1000 times below Canada's 0.1 ppm limit and yielded comparable results to methods involving prior sample preparation. Satisfactory results were obtained for instrument linearity (R≥0996), repeatability (RSD ≤ 20%), and recoveries (91-119%). Subsequently, the method was applied for the targeted (79 pesticides) and non-targeted screenings (1750 compounds, pesticides, and veterinary drugs) of 118 urban honey samples collected from Montreal, Canada in 2021. These honey samples were sourced from two different local beekeepers and gathered from various apiaries and locations within the Montreal region during July 2021. Using the targeted analysis, pesticides were not found in urban honey, and if present, they are present in very low concentration, below LOD and MDLs. This article demonstrates the ability of the new technique to integrate both targeted and non-targeted screenings for detecting pesticide residues in honey. In

addition to the screening approach, the physicochemical properties of the urban honey samples were also evaluated. This involved assessing factors such as moisture content, pH, and electrical conductivity to ensure the quality and consistency of the honey samples. The combination of the non-targeted screening method and physicochemical analysis provided a comprehensive understanding of the quality of the urban honey collected from Montreal, Canada, in 2021.

3.2. Introduction

In recent years, the increasing demand for honey has resulted in the emergence of small-scale urban beekeeping as a promising practice to mitigate the decline of honeybee populations (Peters, 2012). However, the safety of urban honey has become a concern due to the use of pesticides in urban settings. Honeybees can come into contact with pesticides through the nectar and pollen they collect, leading to residues in honey. For instance, several reports have confirmed the presence of pesticides in urban settings (Ping et al., 2022; Racke & Leslie, 1993; Richards et al., 2016; Rippy et al., 2017). Several studies have also reported neonicotinoids in urban honey (Chen et al., 2014; Kavanagh et al., 2021), at levels below the Canadian and European (EU) Maximum Residue Limits (MRLs). Despite the low concentrations, studies have suggested a connection between sublethal neonicotinoid exposure and detrimental health effects in honeybees (Chen et al., 2014; Zhao et al., 2020). During foraging, honeybees are exposed to pollutants deposited on plants and systemic pesticides, which can result in a toxic hive product or hive collapse (Sheldon et al., 2019). To ensure the safety of honey, MRLs have been established for some pesticides in honey, notably in Canada. However, the unsupervised use of pesticides and potential contamination in urban environments calls for continued research and monitoring of pesticide residues in urban honey to safeguard both bees and consumers.

There has been extensive research into pesticide contamination in honey produced in rural regions, but limited studies have been conducted on urban honey. Lambert et al. (2013) conducted an analysis to evaluate the presence of pesticides and veterinary drug residues in honeybee, honey, and pollen samples collected from eighteen apiaries in western France during four different periods in 2008 and 2009. The research outcomes showed that honey among other matrices had the highest levels of contamination by pesticides and veterinary drugs. Also, contrary to the initial hypothesis,

the findings suggested that certain beehives located in urban areas, which were assumed to be exposed less to pesticides, actually displayed elevated levels of contamination when compared to beehives in rural regions (Lambert et al., 2013). Kavanagh et al. employed ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) to analyze urban honey and found that clothianidin and thiacloprid were frequently present in honey collected from urban areas at levels below the EU MRLs. These findings show that exposure to pesticides is not limited to agricultural settings (Kavanagh et al., 2021). In 2018, Jovetić and his colleagues assessed contaminants in urban honey and bee pollen samples using gas chromatography coupled with mass spectrometry (GC-MS). While pesticides were identified in both matrices, specific details regarding the detected pesticides were not provided. Notably, none of the identified pesticide residues exceeded the limit of quantification (LOQ) set by the method, which was 0.01 mg kg^{-1} (Jovetic et al., 2018). The scarcity of research on urban honey is evident in the limited number of studies examining pesticide levels. The existing studies, focusing primarily on specific pesticides, underscore the worrisome gap in our understanding of urban honey contamination. To address this, additional investigations are imperative to thoroughly evaluate and establish the safety of consuming urban honey.

Liquid chromatography-mass spectrometry (LC-MS) is widely used for multi-residue analysis of food samples due to its sensitivity, selectivity, and capacity to analyze a wide range of compounds (Galani et al., 2019; Huang et al., 2019). However, traditional sample preparation methods, such as liquid-liquid extraction (LLE), have significant drawbacks, such as cost, time consumption, and the use of large volumes of toxic organic solvents (Souza Tette et al., 2016). Additionally, traditional purification methods may remove important compounds, such as additional pollutants, chemical markers, metabolites, etc. (Annie von Eyken et al., 2019). To address these issues, a new

approach called direct injection or dilute and shoot has been developed, which rapidly analyzes trace compounds in food and environmental samples (Annie von Eyken et al., 2019). The process involves diluting the samples by employing a combination of acetonitrile and water, and subsequently introducing them directly into an LC-MS system, Typically, this technique is suitable for matrices characterized by low complexity and high analyte concentrations, as exemplified by honey (Gómez-Pérez et al., 2012; Mol et al., 2008; A. von Eyken et al., 2019). For instance, von Eyken et al. developed an HPLC method coupled to QTOF for analyzing veterinary drugs in honey directly without sample preparation. The approach detected spiked compounds at concentrations 20-100 times lower than regulatory limits, with an analysis time of only 45 minutes, and the spiked compounds were recovered at rates ranging from 103-119% (von Eyken & Bayen, 2019). Similarly, Mol et al. investigated the feasibility of utilizing direct injection of honey into LC-MS and found recoveries ranging from 70 to 120% for 136 pesticides, 36 natural toxins, and 86 veterinary drugs (Mol et al., 2008).

Multi -targeted LC-MS/MS is commonly used for simultaneous analysis of pesticides in food samples, but it has limitations as it only detects predefined targeted pesticides and may miss other contaminants (Bauer et al., 2018). Therefore, non-targeted analysis (NTA) has gained popularity as a promising approach for monitoring pesticide residues in food samples (Hayward et al., 2011; Kunzelmann et al., 2018; Picó et al., 2018; Prata et al., 2022). Research on NTA for pesticides in honey is limited, with only a few studies conducted in this area. For instance, von Eyken and Bayen utilized a QTOF analyzer to identify potential contaminants in honey samples from the Canadian market using a suspect list of 43 compounds, identifying four pesticides, two veterinary drugs, and two other contaminants (von Eyken & Bayen, 2019). Similarly, Gómez-Pérez et al. developed a

similar approach using Exactive Orbitrap MS to retrospectively screen pesticides and veterinary drugs' transformation products (Gómez-Pérez et al., 2015). To date, a combination of direct injection with LC-MS for the screening of pesticides in urban honey has not been reported.

The expansion of beekeeping in cities has led to concerns about the potential exposure of honeybees to environmental contaminants. Although pesticides are infrequently and detected at low levels, the lack of studies contributes to these concerns. Analysis methods for identifying and monitoring pesticide residues in urban honey using NTA methods are limited, and more studies are needed to comprehensively address this issue. Therefore, this study developed and validated a rapid method for targeted analysis of pesticide residues in selected urban honey using the dilute-and-shoot solubilization technique coupled with HPLC-Q-TOF-MS. This study aimed to assess the effectiveness of a rapid method for detecting 21 specific pesticides in urban honey that are relevant to beekeeping. The majority of the chosen pesticides were found to be commonly present in urban areas, as reported by previous studies by Nowell (2021) and the U.S. Geological Survey (1999), while some other pesticides were included in the evaluation because of their potential toxicity. The approach was then applied as a non-targeted screening method to detect pesticides in 139 urban honeys collected from Montreal in 2021. This study's innovation lies in implementing direct injection coupled with LC-MS for targeted and non-targeted screening of contaminants in urban honey. Furthermore, this study aims to evaluate the physicochemical characteristics of urban honey produced in Montreal to assess the quality standards of honey produced in cities. The study will assess parameters such as pH, moisture content, electrical conductivity, and color, which are crucial in determining honey's quality and identity.

3.3. Materials and Methods

3.3.1. Chemical and reagents

The native standards, N'-(2,4-Dimethylphenyl)-N-methylformamide Hydrochloride (DPMF) (CAS: 51550-40-4), were purchased from Toronto Research Chemicals (Toronto, ON, Canada). N-(2,4-Dimethylphenyl)formamide (DMF) (CAS: 60397-77-5) was purchased from Sigma-Aldrich (Burlington, MA, United States). A pesticide mix (79 pesticides) was obtained from Agilent Technologies (Santa Clara, CA, USA). Mass-labeled internal standards listed in (**Table 3 1** List of the isotopically labeled standards and their vendors

) were purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada), Cambridge Isotope Laboratories (Tewksbury, MA, USA), Agilent Technologies, Sigma-Aldrich, and Toronto Research Chemicals (North York, ON, Canada). The HPLC-grade solvents, including acetonitrile, water, LC/MS grade formic acid, and methanol, were purchased from Fisher Scientific (Hampton, NH, United States). LC/MS grade ammonium acetate was obtained from Sigma Aldrich. Water employed in the physicochemical experiments underwent deionization via a Milli-Q water purification system to eliminate ionic impurities. All glassware were baked for four hours at 325°C before use.

Compound	**Vendor**
Azoxystrobin-d4	Sigma-Aldrich
Bifenthrin-d5	C/D/N Isotopes
Diuron-d6	Cambridge Isotope Laboratories
Clothianidin-d3	C/D/N Isotopes
Imidacloprid-d4	Sigma-Aldrich
AMPA-$^{13}C_{15}N$-d2	Agilent Technologies
Glyphosate-d2	C/D/N Isotopes
Triclosan-d3	C/D/N Isotopes
Metolachlor-d6	Cambridge Isotope Laboratories
Atrazine-d5	Cambridge Isotope Laboratories

Thiametoxam-d3	C/D/N Isotopes
Carbamazepine-d10	C/D/N Isotopes
Caffeine-d3	Cambridge Isotope Laboratories

3.3.2. Analysis of Pesticides in urban honey

3.3.2.1. Standards & working standards

Table 3 1 List of the isotopically labeled standards and their vendors

All standards were prepared fresh and stored at -20 °C in amber vials. Standards of 10 and 100 mg.mL^{-1} were prepared in acetonitrile. Working standards were prepared from 0.1 to 10 mg.mL^{-1} in ACN by mixing the Agilent pesticide mix, amitraz, DMF, and DPMP. Recovery internal standard (azoxystrobin-d4, bifenthrin-d5, diuron-d6, clothianidin-d3, imidacloprid-d4, AMPA-$^{13}C_{15}ND2$, glyphosate-d2, and triclosan-d3) was prepared in methanol at a concentration of 1 mg/mL. Injection internal standard (metolachlor-d6, atrazine-d5, thiamethoxam-d3, carbamazepine-d10, caffeine-d3), 1 mg.mL^{-1}, were prepared in methanol. Calibration standards were prepared in ACN. Standards were diluted to make seven calibration concentration levels of 0.01,0.05, 0.07, 0.1, 0.2, 0.5, 0.7, 0.5, 1, and 2 ng.mL^{-1}.

3.3.2.2. Honey Samples Collection

A total of one hundred eighteen honey samples were gathered from various apiaries and locations within the Montreal region in July 2021, sourced from two different local beekeepers, for the pesticide analysis. In terms of physicochemical properties, forty-one honey samples were collected from rural areas in Quebec in 2021. These samples were obtained from local stores and were used for comparison with urban samples. They were all unpasteurized and of various types (i.e.,

different floral origins and colors). Approximately 30 mL of each sample was transferred to a stained-glass vial and stored at -20˚C until analysis.

3.3.2.3. Sample preparation

Sample preparation was adapted from von Eyken et al. (A. von Eyken et al., 2019). Around 0.2g of honey was placed in a conical glass tube, and 2 mL of acetonitrile and water (1:1) was added. The samples were then vortexed for about 2 minutes until the honey was wholly dissolved and filtered using a 0.22µm Chrom4 filter. As a final step before injection into the HPLC system, the extract was diluted with water until it constituted 1% honey (w/v) and added 50 µL of a mixture of deuterated internal standards. In this study, although these internal standards were not utilized for quantification purposes, they were intentionally spiked to establish a reference point for sensitivity and retention time. This reference is deemed essential for subsequent non-targeted data analysis in the future (A. von Eyken et al., 2019). The same extraction procedure was followed for the procedural blanks but without any honey samples. In addition, one pooled QC sample was prepared by pooling equal volumes of 10 µL of all extracted real samples and was injected before every 15 samples.

3.3.2.4. Instrumental analysis

Samples were analyzed using an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, USA) coupled with a 6545 quadrupole QTOF-MS (Agilent Technologies, Santa Clara, USA) was utilized to analyze the samples. The instrument was operated using the positive (ESI+) and negative (ESI-) electrospray ionization modes. LC separation was carried out using an InfinityLab Poroshell120 CS-C18 column (2.7µm x 3.0 x 100mm) that was plugged to an

InfinityLab Poroshell 120 CS-C18 guard column (2.7µm × 3.0 mm × 5 mm), both of which were supplied by Agilent Technologies (Santa Clara, USA). For electrospray ionization positive mode, the mobile phase consisted of water (solvent A) and ACN (solvent B), containing 0.1% formic acid. As for the mobile phase for electrospray ionization, the negative mode consisted of water (solvent A) and ACN (solvent B), containing 5 mM ammonium acetate. The gradient profile of the mobile phase was as stated below: 0.2 min 5% B, from 0.20 to 4 min gradient to 100% B, from 4 to 6 min 100% B, from 6 to 6.10 min 100% B and from 6.10 min to 9 min 5% B. The flow rate was at 0.3 mL/min, the injection volume was at 20 µL, and the column temperature was set at 20 °C. The drying gas temperature was 275°C with a flow of 10 L/min, sheath gas temperature was 325°C with a flow of 12 L/min, the pressure on the nebulizer was 30 psig, the capillary voltage was 4000 V, the fragmentor voltage was 125 V, the skimmer voltage was 65 V and the nozzle voltage was 250 V. MS/MS data for all ions was gathered by conducting MS scans with a scan rate of 2 spectra/s, with the range of m/z 70 to 1700. To prevent any contamination, the first 2.5 minutes of the elution were discarded. The samples were stored in the multi-sampler compartment at a temperature of 4°C.

Figure 3 1 Honey samples' preparation for analysis. Method adapted from (von Eyken et al., 2019).

3.3.2.5. Method validation

Three honey samples were selected without detectable amounts of target pesticides (S036, S109 & and 123). All three samples were preliminarily tested for 95 pesticides using targeted screening,

and the results indicated that none of the pesticides were detected in the honey samples. The samples utilized in the study were sourced from diverse floral and geographical origins, exhibiting variations in both color and farming techniques. This approach was undertaken to encompass the wide range of matrices and their inherent variability within the study. For these reasons, these samples were used as matrix blanks for the method validation. For recovery and linearity tests, three replicates of each blank matrix were prepared and spiked with the native pesticide standard's mix to achieve different target concentrations in honey ranging from 0.01 to 2 $\mu g.g^{-1}$. Using previously detected levels in honey as a reference, the spiking levels were chosen according to the lowest, mid, and highest concentrations. The matrix effect was evaluated by injecting the native pesticide standard directly into the 1% honey sample prepared for LC-QTOF analysis. The injected samples included 9 different concentration levels of the pesticide, ranging from 0.1 to 2 $ng.mL^{-1}$, which corresponded to a concentration range of 0.001 to 0.2 $ug.g^{-1}$ in honey. Two procedural blanks were prepared with the samples and analyzed several times throughout the analysis, after every 15 samples, and use to derive the method detection limit (MDL) (3σ).

3.3.3. Physicochemical properties of urban honey

All one hundred eighteen samples' physicochemical properties were analyzed. Before analysis, samples were removed from – 20 °C freezers and kept at room temperature for around an hour or until all the honey samples were completely defrosted. Physicochemical parameters of honey, such as moisture, ash, and electrical conductivity, were examined using standards methods recommended by the International Honey Commission (Bogdanov et al., 2002).

3.3.3.1. Color

In Canada, the color of honey is commonly measured using the Pfund color grading system, which is based on the intensity of light transmission through a honey sample. The Pfund color scale is widely used in the honey industry in Canada and is a standardized method for measuring honey color. Since various analytical tools can be used to classify the color of honey samples by producers, Jack's scale, the method recommended by the Canadian Food Inspection Agency (CFIA), was used in this study. This method relies on analyzing the honey samples directly without any further dilution. Jack's scale depends on a visual comparison between the honey sample and Jack's scale chart, where the results are expressed in (mm) Pfund.

3.3.3.2. Moisture Content

Moisture content was determined using Abbe's refractometer. The digital refractometer was regularly calibrated with distilled water and cleaned with ethanol between samples. The protocol was adapted from (Bogdanov et al., 2002).

3.3.3.3. pH

Using an Oakton PC 700 pH/Conductivity meter, the pH of a 13.3 % (w/v) solution of honey prepared in milli-Q water was determined (Bogdanov et al., 2002). The pH meter was regularly calibrated at pH 4, 7, and 10.

3.3.3.4. Electrical conductivity (EC)

Conductivity measurements were performed using a conductivity meter, an Oakton PC 700 pH/Conductivity meter, and a 20% (w/v) honey solution suspended in Milli-Q water.

3.3.4. Statistical analysis

The concentrations of pesticides were determined by employing the Agilent Mass Hunter Workstation Software - Quantitative Analysis B.07.01. This was done using a m/z extraction window value ± 20 ppm. For each compound, the matrix effect, recovery, instrument linearity, method linearity, repeatability, instrument detection limit (IDL), MDL, and limit of quantification (LOQ) were determined. The linearity of instrument response was determined for every compound by measuring the relative standard deviation (RSD) of the response factors (RF) of nine calibration curve standards, which ranged from 0.1 to 2 $ng.mL^{-1}$. Method precision was evaluated by performing repeatability tests which was studied by analyzing replicates of spiked matrix blank samples at different fortification levels extracted on the same day. Trueness was estimated in terms of recovery by evaluating different spiking concentration levels of standards. As for matrix effect assessment (ME), it was determined by comparing the matrix-matched standards to solvent standards. ME (%) is calculated using the equation [(slope of standards in matrix-slope of standards in solvent/slope of standards in solvent) × 100] (Souza Tette et al., 2016). The method's overall linearity was evaluated by calculating the Pearson coefficient of the linear correlation between the theoretical spiked concentrations and the experimental results. To evaluate repeatability, three honey samples were spiked with a known amount of analyte, and the relative standard deviation (RSD) was calculated. The IDL was established by identifying the concentration at which the signal-to-noise ratio (S/N) reached 3, calculated based on the S/N of the lowest standard of the calibration curve. The MDL was calculated based on the signals obtained from the repeated measurements (n=3) of the lowest calibration standard in matrix, which were analyzed around the retention time of each compound. Specifically, we calculated MDL as three

times the standard deviation (σ) of the signals obtained from the procedural blanks. Finally, the LOQ was calculated as 3.3 times the MDL.

The method's (matrix effect, recovery, repeatability, method linearity, and MDL) performances for the different honey colors values were compared through two-way analysis of variance (ANOVA) tests.

Data analysis and visualization for physicochemical results were performed in Python 3.10.9 using the following libraries: matplotlib 3.6.2,(Hunter, 2007) numpy 1.23.5, (Ozoani et al., 2020) pandas 1.5.2,(McKinney, 2010) scikit-learn 1.2.1,(Pedregosa et al., 2012) and scipy 1.10.0.(Virtanen et al., 2020). The hue of the decision boundary at any given point is determined by the number of rural and urban samples found in the 7-nearest neighbors to that point and ranges from yellow (urban) to violet (rural). Values in brackets indicate the explained variance ratio of each principal component. Arrows show the distance and direction a sample would move in if that feature were to increase in value by one standard deviation while all other features remained constant.

3.4. Results and discussion

3.4.1. Physicochemical properties

The findings of this study involved a comparative evaluation of urban honey specimens (n=138) with those of rural origin collected from Quebec (n= 41). The assessments of the rural honey specimens were conducted by fellow researchers. The origin of the nectar in rural and urban honey remains uncertain, and it is not known whether it is derived from blossoms or honeydew. **Table 3 *2*** displays the summary of the physicochemical characteristics of the analyzed honeys. The

moisture content of honey is influenced by the prevailing climate conditions, and this parameter is a crucial determinant of its shelf life, stability, and ability to resist spoilage from yeast fermentation during storage (Matović et al., 2018). The range of moisture content observed was between 16.00 ± 1.13 for urban honey and 15.42 ± 2.1 for rural honey. All honey samples were all below 20%, the maximum value established by the Codex standard for honey. The moisture content of urban honey reported here is similar to those recorded by Preti et al. (2021)(average of 15.18%).

Analysis of all urban honey samples revealed acidic nature, with a pH range of 3.7 to 4.7. These values are consistent with the average pH range of 1000 honey samples analyzed worldwide (Solayman Md et al., 2016).

The analysis of the color distribution in the urban honey sample revealed that the majority of the honeys was white (46.04%) or golden (42.45%) in color. The higher proportion of white and golden honey in this sample could be attributed to the floral sources available in urban environments, which may differ from those found in rural or wild areas. The relatively low percentage of amber (10.79%) and dark (0.72%) honey in this sample could also reflect the types of plants available in the urban environment, as darker honey is often associated with plants such as buckwheat or black locust (Kuś et al., 2014) that may not be as prevalent in urban areas. Overall urban honey had a color that ranged between 0 and 90 mm. These results are different than previous studies that have found honey from urban areas to be generally darker with a range of 71.42-158.14 mm (Kavanagh et al., 2019; Preti & Tarola, 2021) compared to honey from rural or wild areas.

The honey samples collected from urban areas had a mean EC of 0.36 ± 0.11 mS/cm. EC serves as a valuable tool for identifying the botanical and geographical origin of honey, as well as its type, such as blossom honey or honeydew (Thrasyvoulou et al., 2018). The level of EC is influenced by the ash and acid content of honey, which is in turn affected by the mineral content of the soil

(Kavanagh et al., 2019). Most of the samples tested exhibited EC values within the standard limit of less than 0.8 mS/cm (The Codex Alimentarius, 2019) , except for two samples, which had values of 0.84 and 0.86 mS/cm, respectively. The range of EC values observed in the samples was between 0.11 and 0.67 mS/cm. Based on these findings, it can be concluded that all the samples analyzed were blossom honey, except for two samples which had EC values above the standard limit, as noted above.

Honey type	**EC ($mS.cm^{-1}$)**	**pH**	**Moisture**	**Color**			
				White (%)	Golden (%)	Amber (%)	Dark (%)
Urban (n=139)	0.36 ± 0.11	4.13 ± 0.22	16.00 ± 1.13	46.04	42.45	10.79	0.72
Rural (n=48)	0.26 ± 0.09	4.13 ± 0.29	15.42 ± 2.11	60	23	17	0

Table 3 2 Physicochemical parameters of urban and rural honey samples from Quebec, Canada

3.4.1.1. Physicochemical properties urban vs rural

A Kruskal–Wallis H test was performed, revealing a significant difference between the EC of urban and rural honey ($p=3.60\times10^{-8}$), with the mean conductivity of urban honey (0.359 mS/cm) being 38% higher than the mean conductivity of rural honey (0.261 mS/cm). No significant differences were found between urban and rural honey based on colour (p=0.500), pH (p=0.783), or water content (p=0.275). These results are readily visible in (**Figure 3** ***2***) whereby conductivity (the only feature to show a significant difference between rural and urban honey) is almost perfectly aligned with PC1 (the principal component that captures the most variance). It follows that samples found at a higher PC1 value also tend to be urban honey due to their higher conductivity. These findings indicate that urban honey from Quebec meets acceptable quality

standards and is comparable to rural honey in terms of pH, moisture, and color. This is important for ensuring consumer confidence in the safety and quality of urban honey. Secondly, the results highlight the limitations of relying solely on physicochemical properties to differentiate between urban and rural honeys. This emphasizes the need for additional analytical methods to better characterize and differentiate honey samples. Chemical fingerprinting studies, such as those employing techniques like LC-MS, can provide a more comprehensive analysis of the chemical composition of honey, including the presence of specific compounds or markers that can help differentiate between urban and rural sources. Thus, the findings of this study suggest that while urban and rural honey can be differentiated based on conductivity, this feature alone is insufficient to classify the type of honey reliably.

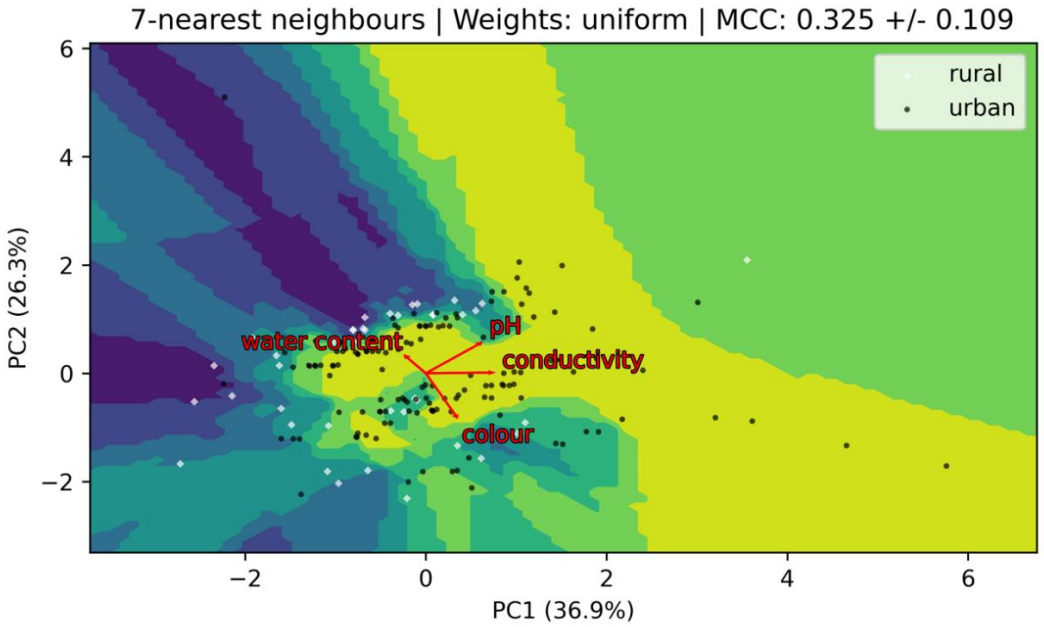

Figure 3 2 Decision boundary biplot of the first two principal components based on the physicochemical analysis of rural (n=41) and urban (n=138) honey samples.

These findings represent the initial dataset describing urban honey in Montreal. However, asserting that the examined urban honey samples are representative of all urban honey in Montreal proves

challenging due to several factors. The study involved 138 samples, and the precise origin of the honey, whether derived from blossom or honeydew, remains uncertain, which can significantly influence its physicochemical characteristics. Nevertheless, this study is the first study to provide insights into the physicochemical properties of urban honey, which can be compared to those of rural honey samples collected from Quebec. The research discovered that urban honey had a higher EC compared to rural honey, but no significant differences were noted between urban and rural honey based on color, pH, or water content. It's worth noting that the results may vary in different urban areas with diverse environmental conditions and floral sources available. Therefore, further studies with larger sample sizes and a wider geographical distribution are necessary to draw more general conclusions about the physicochemical properties of urban honey.

3.4.2. Method validation

Table 3 3 to **Table 3 7** provide various parameters for 21 target analytes analyzed using the electrospray ionization positive (ESI+; 16 pesticides) and negative (ESI-; 5 pesticides) for every color honey sample. The linearity of the instrument was evaluated by calculating the RSD values of the RF of the calibration curve standards. The RSD values were generally below 20%, indicating good linearity, with the exception of carbaryl, which had an RSD value of 22.6%. Despite the high RSD value for carbaryl, the instrument still met SANCO's guidelines. IDLs varied between 0.01 and 0.5 ng. mL^{-1} for positive and negative electrospray ionization.

3.4.2.1. Matrix effects

The matrix effects (ME) observed for the different honey colors were not significantly different based on ANOVA in both positive mode (P=0.97) and negative mode (P=0.99). Based on the formula, a 100% value indicates no ME. A value lower than 100% implies matrix suppression,

while a greater than 100% indicates matrix enhancement. Typically, MEs are classified as mild when their values range from 80% to 120%. Medium MEs are values between 50% and 80% or 120% and 150%. Strong MEs wre observed when the values are below 50% or above 150% (Kmellár et al., 2008). In this study, most MEs were classified as mild or medium, with only a few exceptions where no or strong MEs were observed. Specifically, naled and fipronil were exceptions, as no MEs were recorded for these two compounds. The ME of fipronil was similar to what was reported by García-Chao et al. (2010), where they used a Doehlert experimental design to optimize the extraction of target compounds from raw hives (García-Chao et al., 2010). A strong matrix enhancement effect was observed for imidacloprid (average ME = 155%). This is consistent with the findings reported by Hou et al., for imidacloprid in honey, who used traditional solid-phase extraction (SPE) (Hou et al., 2019). However, in another study that also used SPE, Gbylik-Sikorska et al. reported a lower average signal enhancement (Gbylik-Sikorska et al., 2015). In summary, the investigation determined that ME differed across compounds rather than different honey samples. This implies that the pesticide's properties primarily influence the MEs. Therefore, using an average ME can effectively correct the matrix effects, but it is advisable to evaluate the effects for each compound.

3.4.2.2. Method detection limit and limit of quantification

The study found no significant difference between the minimum MDLs and LOQs of positive and negative mode when using honey colors, with p-values of 1 and 0.11 for MDLs, and 0.54 and 0.87 for LOQs, respectively. The MDLs ranged from 0.0001 to 0.048 $\mu g.g^{-1}$ in honey, indicating that pesticides can be detected at levels as low as 0.02 pg of pesticides injected. The LOQ for positive compounds ranged from 0.0004 to 0.09 $\mu g.g^{-1}$, while for negative compounds, it varied between

0.0006 and 0.003 $\mu g.g^{-1}$. These MDLs and LOQs are appropriate as they are lower than the regulatory limit (MRLs) in Canada, which is 0.1 ppm for various pesticide residues (Government of Canada, 2022). Souza Tette et al. measured 116 pesticides in honey using the QuEChERS method with LC-MS/MS and reported MDLs of 0.005 $\mu g.g^{-1}$ and LOQs ranging from 0.01 to 0.025 $\mu g.g^{-1}$ (Tette et al., 2016). In the latter study, diazinon and methiocarb had a higher MDL of 0.005 and LOQ of 0.01 $\mu g.g^{-1}$ compared to the MDL of 0.001 and 0.0007 $\mu g.g^{-1}$, and LOQ of 0.002 and 0.004 $\mu g.g^{-1}$, respectively, in our study. Zheng et al. reported LOQs of 0.001-0.005 $\mu g.g^{-1}$ for simultaneously quantifying pesticide residues in honey using a modified QuEChERS extraction coupled to LC-MS/MS (Zheng et al., 2018). Conversely, Almeida et al. optimized and validated a method for identifying and quantifying pesticides in honey and reported comparable LOD and LOQ ranges of 0.001-0.0004 $\mu g.g^{-1}$ and 0.0002 - 0.0008 $\mu g.g^{-1}$, respectively (Almeida et al., 2020). Therefore, the present findings suggest that the pesticide residues in honey can be detected using the dilute and shoot approach at concentrations similar and sometimes lower than those reported in previous studies that used conventional extraction methods.

3.4.2.3. Recovery (Trueness)

All the recovery percentages fell within the acceptable range of 91-120%, except for the recovery of Fludioxonil in white honey (123%). However, the overall average recovery of Fludioxonil (118 %) falls within the acceptable ranges. The results showed a substantial increase in recoveries at very low spiking levels (0.01 ng. mL^{-1}), potentially indicating the influence of high spiking levels. As these high levels were having an impact on the overall outcomes, they were excluded from the results. ANOVA showed no significant difference in recoveries between positive and negative pesticides for different honey colors (P=0.27 and P=0.997, respectively). These results align with

previous findings reported by other researchers studying pesticides in honey, where recoveries are typically observed to fall between 80-120%, with some exceptions that exceed or fall below this range (Blasco et al., 2003; Hrynko et al., 2018; Pirard et al., 2007; Tanner & Czerwenka, 2011; Tette et al., 2016).

3.4.2.4. Repeatability

The precision was determined as the percentage of relative standard deviation (% RSD), which can be observed in **Table 3 3** to **Table 3 7**. The findings indicate that all results demonstrated an average RSD below 10%, except for propiconazole and diazinon (13 and 20%, respectively). While there was no significant difference in repeatability among the different honey colors based on the statistical analysis (ANOVA, P=0.07 and 0.69 for positive and negative), a noticeable variation was observed in each compound among the different honey colors, reaching up to 39%. It can be concluded that although the method demonstrated good overall precision, certain compounds exhibited more significant variability across different honey colors, emphasizing the importance of considering honey color during compound analysis for reliable and accurate results.

3.4.2.5. Method linearity

Based on the chemicals evaluated, linearity was observed throughout the concentration range investigated, with Pearson coefficients R ranging from 0.994 and 0.999 for method linearity. These high correlation coefficients demonstrate a strong linear relationship between the concentration of the compounds and the corresponding response values. Additionally, the investigation found that the values of all three honey colors for each of the compounds were not significantly different in both positive and negative modes (based on ANOVA with p-values of 0.77 and 0.78, respectively),

indicating that method linearity was consistent across all honey samples tested. As a result of this, the method is regarded as linear across the proposed working range.

Parameter	**Naled**	**Atrazine**	**Methiocarb**	**DEET**
	m/z **379.2603**	m/z **216.101**	m/z **226.1341**	m/z **192.1382**
	RT[d]= 4.16 min	**RT=4.7**	**RT= 4.88**	**RT=4.64**
Instrument linearity (RSD % of RF)	6.6	11.3	7.2	13.6
IDL(ng.mL $^{-1}$)	0.01	0.01	0.01	0.01
Matrix effect (%)[a]	W[a]: 101.45 ± 7.45 D[b]: 100.40 ± 7.55 G[c]: 103.44 ± 8.73	W: 94.37 ± 14.50 D: 97.90 ± 18.61 G: 99.57 ± 24.65	W: 86.88 ± 10.44 D: 85.71 ± 10.85 G: 88.89 ± 9.97	W: 98.88 ± 10.80 D: 95.82 ± 5.37 G: 90.65 ± 6.60
MDL (µg.g^{-1} honey)	W: 0.0001 D: 0.00001 G: 0.0002 Average = 0.0001	W: 0.0004 D: 0.0007 G: 0.0001 Average = 0.0004	W: 0.0009 D: 0.0009 G: 0.0004 Average = 0.0007	W: 0.001 D: 0.00003 G: 0.0002 Average = 0.0004
LOQ (µg.g^{-1} honey)	W: 0.0003 D: 0.0001 G: 0.0007 Average= 0.0004	W: 0.001 D: 0.002 G: 0.0005 Average= 0.001	W: 0.003 D: 0.003 G: 0.001 Average= 0.002	W: 0.005 D: 0.0001 G: 0.0007 Average= 0.002
Recovery (%)	W: 97.39 ± 11.26 D: 91.86 ± 4.54 G: 104.87 ± 7.77	W: 101.18 ± 10.61 D: 99.70 ± 3.93 G: 105.28 ± 19.18	W: 102.59 ± 9.30 D: 109.63 ± 6.26 G:106.09 ± 9.15	W: 101.92 ± 8.02 D: 103.05 ± 15.33 G: 106.85 ± 24.93
Method linearity (R)	W: 0.998 D: 0.999 G: 0.998	W: 0.998 D: 0.996 G: 0.998	W: 0.995 D: 0.998 G: 0.997	W: 0.995 D: 0.998 G: 0.996
Repeatability (RSD %)	W: 1.35 D: 1.82 G: 1.92	W: 5.33 D: 4.75 G: 3.69	W: 2.59 D: 2.66 G: 1.69	W: 4.56 D: 3.06 G: 4.1

Table 3 3 Method performance for the sixteen targeted pesticides for m/z extraction window of ± 20 ppm, using ESI +, in three different honey color

[a]W= White, D[b]=Dark, G[c]=Golden, RT[d]= Retention time

Parameter	Primicarb	Dodemorph	Propiconazole	Diazinon
	m/z **239.1534**	m/z **282.2792**	m/z **342.0771**	m/z **305.1089**
	RT= 3.19	**RT= 3.613**	**RT= 5.42**	**RT= 5.677**
Instrument linearity (RSD % of RF)	8.5	10.2	15.8	8.9
IDL(ng.mL $^{-1}$)	0.01	0.01	0.05	0.01
Matrix effect (%)[a]	W: 63.36 ± 5.33 D: 71.63 ± 5.64 G: 71.63 ± 5.00	W: 91.66 ± 11.13 D: 94.52 ± 11.13 G: 91.73 ± 11.01	W: 87.90 ± 18.10 D: 88.69 ± 20.57 G: 91.57 ± 26.51	W: 83.39 ± 10.75 D: 74.70 ± 19.82 G: 75.93 ± 29.56
MDL (µg.g^{-1} honey)	W: 0.001 D: 0.0003 G: 0.0003 Average = 0.0005	W: 0.0005 D: 0.0003 G: 0.0003 Average = 0.0004	W: 0.002 D: 0.003 G: 0.003 Average = 0.003	W: 0.002 D: 0.002 G: 0.0002 Average = 0.001
LOQ (µg.g^{-1} honey)	W: 0.005 D: 0.001 G: 0.001 Average= 0.002	W: 0.002 D: 0.001 G: 0.001 Average= 0.001	W: 0.01 D: 0.01 G: 0.01 Average= 0.01	W: 0.006 D: 0.005 G: 0.001 Average= 0.004
Recovery (%)	W: 115.40 ± 30 D: 111.04 ± 16.94 G: 118.48 ± 9.0	W: 115.33 ± 29.54 D: 107.97 ± 19.68 G: 116.86 ±13.03	W: 103.79 ± 12.24 D: 102.34 ± 4.24 G: 102.35 ± 3.74	W: 102.00 ± 6.27 D: 97.05 ± 9.77 G: 108.93 ± 12.86
Method linearity (R)	W: 0.999 D: 0.997 G: 0.999	W: 0.999 D: 0.999 G: 0.998	W: 0.994 D: 0.997 G: 0.998	W: 0.998 D: 0.998 G: 0.995
Repeatability (RSD %)	W: 4.607 D: 2.14 G: 2.99	W: 1.82 D: 2.27 G: 0.60	W: 13.10 D: 19.19 G: 6.33	W: 39.59 D: 17.00 G: 2.82

Table 3 4 Method performance for the sixteen targeted pesticides for m/z extraction window of ± 20 ppm, using ESI +, in three different honey color

Parameter	Imidacloprid	Diuron	Carbaryl	Malathion
	m/z **256.0603**	m/z **233.0241**	m/z **202.0865**	m/z **331.0435**
	RT[d]= 3.84	**RT= 4.73**	**RT= 4.64**	**RT=5.27**
Instrument linearity (RSD % of RF)	12.7	10.2	22.6	14.5
IDL(ng.mL $^{-1}$)	0.07	0.2	0.25	0.2
Matrix effect (%)[a]	W[a]: 161.00 ± 45.74 D[b]: 150.19 ± 54.81 G[c]: 155.81 ± 50.58	W: 92.97 ± 8.93 D: 97.57 ± 14.94 G: 95.79 ± 19.03	W: 85.29 ± 22.85 D: 115.16 ± 40.85 G: 92.26 ± 43.42	W: 103.62 ± 23.81 D: 94.68 ± 22.77 G: 109.69 ± 21.74
MDL (µg.g^{-1} honey)	W: 0.006 D: 0.009 G: 0.005 Average = 0.006	W: 0.007 D: 0.005 G: 0.01 Average = 0.007	W: 0.023 D: 0.043 G: 0.048 Average = 0.035	W: 0.022 D: 0.027 G: 0.015 Average = 0.021
LOQ (µg.g^{-1} honey)	W: 0.020 D: 0.029 G: 0.016 Average= 0.02	W: 0.022 D: 0.018 G: 0.030 Average= 0.023	W: 0.08 D: 0.14 G: 0.16 Average= 0.126	W: 0.073 D: 0.089 G: 0.050 Average= 0.070
Recovery (%)	W: 94.81 ± 18.29 D: 99.58 ± 7.28 G: 102.40 ± 14.96	W: 100.73 ± 5.12 D: 99.97 ± 5.13 G: 99.64 ± 3.76	W: 99.99 ± 0 D: 103.58 ± 2.90 G:99.08 ± 1.65	W: 100.60 ± 12.25 D: 96.68 ± 7.50 G: 104.21 ± 11.10
Method linearity (R)	W: 0.996 D: 0.998 G: 0.996	W: 0.998 D: 0.996 G: 0.998	W: 0.976 D: 0.986 G: 0.982	W: 0.996 D: 0.990 G: 0.998
Repeatability (RSD %)	W: 7.5 D: 6.6 G: 2.6	W: 5.37 D: 5.20 G: 3.6	W: 10.66 D: 6.69 G: 3.79	W: 15.10 D: 4.77 G: 11.66

Table 3 5 Method performance for the sixteen targeted pesticides for m/z extraction window of ± 20 ppm, using ESI +, in three different honey color

Parameter	DPMF	DMF	Clothianidin	Thiamethoxam
	m/z **163.1242**	m/z **150.0913**	m/z **250.0154**	m/z **292.0264**
	RT= 2.80	**RT= 4.19**	**RT= 3.78**	**RT= 3.55**
Instrument linearity (RSD % of RF)	9.7	6.7	11.9	18.2
IDL(ng.mL $^{-1}$)	0.05	0.5	0.1	0.05
Matrix effect (%)[a]	W: 104.57 ± 15.55 D: 103.88 ± 12.11 G: 111.64 ± 18.91	W: 103.38 ± 15.58 D: 108.57 ± 10.15 G: 114.82 ±14.35	W: 97.00 ± 19.65 D: 99.47 ± 14.82 G: 103.16 ± 13.99	W: 150.21 ± 27.53 D: 160.90 ± 38.43 G: 123.96 ± 56.35
MDL (μg.g^{-1} honey)	W: 0.001 D: 0.001 G: 0.002 Average = 0.001	W: 0.027 D: 0.009 G: 0.026 Average = 0.021	W: 0.015 D: 0.005 G: 0.006 Average = 0.009	W: 0.003 D: 0.003 G: 0.002 Average = 0.003
LOQ (μg.g^{-1} honey)	W:0.005 D: 0.005 G: 0.007 Average= 0.006	W:0.089 D: 0.029 G: 0.085 Average= 0.068	W:0.05 D: 0.02 G: 0.02 Average= 0.03	W: 0.01 D: 0.01 G: 0.01 Average= 0.01
Recovery (%)	W: 110.28 ± 19.83 D: 106.26 ± 7.76 G: 103.67 ± 10.57	W: 107.15 ± 9.04 D: 98.01 ± 4.38 G: 99.30 ±9.92	W: 100.79 ± 6.95 D: 99.77 ± 7.38 G: 98.31 ± 3.70	W: 96.79 ± 4.21 D: 97.57 ± 9.94 G: 100.99 ± 7.16
Method linearity (R)	W: 0.999 D: 0.999 G: 0.999	W: 0.995 D: 0.996 G: 0.995	W: 0.994 D: 0.996 G: 0.998	W: 0.997 D: 0.999 G: 0.998
Repeatability (RSD %)	W: 4.09 D: 4.33 G: 1.65	W: 4.78 D: 7.67 G: 2.23	W: 7.99 D: 8.55 G: 1.92	W: 6.90 D: 6.89 G: 0.07

Table 3 6 Method performance for the sixteen targeted pesticides for m/z extraction window of ± 20 ppm, using ESI +, in three different honey color

Parameter	Benzovindiflupyr	Fipronil	Chlorfenapyr	Fludioxonil	Teflubenzuron
	m/z **396.0484**	m/z **434.9307**	m/z **347.1270**	m/z **247.0319**	m/z **378.9664**
	RT[d]= 5.48	**RT= 5.41**	**RT= 5.08**	**RT= 5.09**	**RT= 5.89**
Instrument linearity (RSD % of RF)	7.8	9.5	6.5	9.4	11.6
IDL (ng.mL ^{1})	0.01	0.01	0.05	0.01	0.05
Matrix effect (%)	W[a]: 81.31 ± 8.03 D[b]: 78.67 ± 6.63 G[c]: 76.38 ± 6.79	W: 100.56 ± 6.59 D: 102.22 ± 9.26 G: 99.82 ± 6.78	W: 53.71 ± 6.10 D: 56.09 ± 5.03 G: 54.85 ± 5.02	W: 64.50 ± 6.80 D: 61.03 ± 5.48 G: 70.79 ± 6.14	W: 74.08 ± 10.21 D: 82.85 ± 10.93 G: 73.68 ± 12.23
MDL (µg.g^{-1} honey)	W: 0.0001 D: 0.0003 G: 0.0003 Average= 0.0002	W: 0.0003 D: 0.0002 G: 0.00001 Average= 0.0002	W: 0.0008 D: 0.0005 G: 0.0008 Average= 0.0007	W: 0.0002 D: 0.0001 G: 0.0002 Average= 0.0002	W: 0.004 D: 0.002 G: 0.003 Average= 0.003
LOQ (µg.g^{-1} honey)	W: 0.0002 D: 0.001 G: 0.001 Average= 0.0007	W: 0.001 D: 0.001 G: 0.001 Average= 0.001	W: 0.003 D: 0.002 G: 0.003 Average= 0.003	W: 0.0008 D: 0.0005 G: 0.0005 Average= 0.0006	W: 0.012 D: 0.006 G: 0.009 Average= 0.009
Recovery (%)	W: 106.77 ± 15.51 D: 99.55 ± 1.35 G: 104.95 ± 9.23	W: 116.41 ± 26.47 D: 103.81 ± 7.45 G: 102.11 ± 5.51	W: 103.48 ± 18.41 D: 104.38 ± 18.41 G: 110.21 ± 7.97	W: 123.04 ± 23.93 D: 119.50 ± 5.82 G: 111.23 ± 13.00	W: 107.19 ± 1.28 D: 93.97 ± 7.68 G: 113.50 ± 18.25
Method linearity (R)	W: 0.998 D: 0.999 G: 0.998	W: 0.998 D: 0.998 G: 0.998	W: 0.998 D: 0.997 G: 0.998	W: 0.998 D: 0.999 G: 0.996	W: 0.999 D: 0.997 G: 0.999
Repeatability (RSD %)	W: 4.71 D: 3.48 G: 3.44	W: 1.68 D: 1.41 G: 0.92	W: 3.43 D: 1.36 G: 6.41	W: 2.80 D: 1.30 G: 7.45	W: 4.66 D: 9.30 G: 4.99

Table 3 7 Method performance for the five targeted pesticides for m/z extraction window of ± 20 ppm, using ESI -, in three different honey color

3.4.1. Targeted screening

The optimized method was employed to analyze 139 urban honey samples collected from Montreal in 2021. None of the 79 targeted pesticides were detected in the urban honey, indicating their concentrations were below 0.0001 $\mu g.g^{-1}$ based on the individual MDLs. These findings differ from previous studies on urban honey. In a study by Kavanagh et al. (2021), which focused on 10 Irish honey samples from urban environments analyzed using UHPLC-MS, clothianidin was among the most commonly detected neonicotinoids, but at levels below 0.05 $mg.kg^{-1}$. Another study conducted in 2018 examined honey and bee pollen samples from the Belgrade metropolitan area and detected 123 pesticides, including diazinon, carbaryl, and primicarb, among others (Jovetic et al., 2018). Conversely, all these pesticides were found to be below the LOQ of 0.01 $ng.g\text{-}^{1}$. However, the absence of detected pesticides doesn't guarantee the absence of any residues, such as other active ingredients or degradation products. The targeted screening was designed to detect a specific set of pesticides, and if other pesticides were present, they would not be detected by this method. Therefore, non-targeted screening is necessary to identify any other pesticides that may be present in the samples.

3.4.2. Non-targeted screening

The main objective of this study was to detect and identify pesticides present at trace levels in urban honey, using a non-targeted approach, which is a novel method for studying urban honey. Previous work had demonstrated the capacity of this approach to accurately identify contaminants in honey at trace levels, and the methodology was optimized accordingly (von Eyken & Bayen, 2019).

For the analysis, 125 non-spiked honey samples were screened, along with pooled quality control samples and blanks, using MassHunter Profinder B.10.00 software. The data was processed using the "*Targeted Feature Extraction*" mode, which involved aligning peaks, extracting molecular features, and comparing the resulting information with a database. The MassHunter Pesticides PCDL library, containing 1750 compounds, was utilized for analysis.

In total, 111 compounds (Table 3 9) were tentatively identified in at least one honey sample with a matching score above 80%. It's important to note that further extensive studies are needed to confirm the presence of these compounds. Among the significant findings, maleic hydrazide (score of 96%) received one of the highest scores and has been linked to genotoxic effects and tumor induction (EPA, 1994), yet the EPA's <15 ppm threshold in technical-grade products eliminates worries of lasting cancer risks for humans through diet and occupational exposure (EPA, 1994). Some highly scored pesticides, including the fungicide kresoxim methyl, have previously been detected in honey, particularly commercial honey in Brazil, exceeding the maximum residue limit but remaining below the LOQ (0.01 $\mu g.g^{-1}$) using a modified QuEChERS method coupled with gas chromatography and electron capture detection. On the other hand, certain potential compounds like metolcarb, commonly used to control pests in agricultural settings such as rice leafhoppers, plant hoppers, and fruit flies (Yang et al., 2015), have not yet been detected in honey. However, they have been reported in other food sources, such as fruits and vegetables (Kmellár et al., 2008).

It is noteworthy that very limited studies have utilized a non-targeted approach for honey analysis, particularly in the context of urban honey. To the best of our knowledge, this is the first study to employ a non-targeted approach to analyze urban honey. By adopting this novel method, we have

provided valuable insights into the presence of pesticides in urban honey, shedding light on potential contaminants and offering a new perspective on the safety and quality of urban honey.

Parameter	Value
Match mass tolerance	±5 ppm
Peak filter (absolute height)	≥ 200 counts
Expansion values for chromatogram extraction (*m/z*) (+/-)	10 ppm
Isotope abundance score	60%
Limit EIC extraction range (expected RT +/-)	1.5 min
Limit to the largest 2000 features	Not selected
Score filter: "don't match when < 70" and "do not match if the unobserved second ion's abundance is expected to be > 200"	Not selected
Ion and adducts considered	+H for positive ion -H for negative ion
Integrator method	Agile 2
Peak spectra: spectra to include how much percent of average scan	>10%
TOF spectra: exclude if above how much saturation	>20%
Post processing: Find by formula peak filter (absolute height)	≥1000 counts

Table 3 8 Parameters for feature extraction

Name	Formula	Mass (avg)	RT (avg)	Score (%)

Kresoxim-methyl	$C_{18}H_{19}NO_4$	313.1317	2.85	98.8
Ascaridole	$C_{10}H_{16}O_2$	168.1156	3.69	97.1
Ethephon	$C_2H_6ClO_3P$	143.975	5.48	96.9
Quinacetol	$C_{11}H_9NO_2$	187.0638	2.91	96.9
Demeton-S-methylsulfoxide	$C_6H_{15}O_4PS_2$	246.0138	2.28	96.1
Maleic hydrazide	$C_4H_4N_2O_2$	112.0274	2.17	96
Triazbutil	$C_6H_{11}N_3$	125.0953	7.84	95.3
Difenoxuron	$C_{16}H_{18}N_2O_3$	286.1318	2.82	95
2,6-Xylidine (2,6-Dimethylaniline) (Lidocaine-M)	$C_8H_{11}N$	121.0896	1.79	93.9
Fenobucarb (Baycarb)	$C_{12}H_{17}NO_2$	207.1267	2.9	93.7
8-Hydroxychinolin (8-Hydroxyquinoline)	C_9H_7NO	145.0534	3.06	93.3
Metolcarb	$C_9H_{11}NO_2$	165.0802	2.65	93
Mecarbam	$C_{10}H_{20}NO_5PS_2$	329.051	2.54	92.9
Sulfadimidine (Sulfamethazine)	$C_{12}H_{14}N_4O_2S$	278.0832	1.89	92.7
Isoprocarb	$C_{11}H_{15}NO_2$	193.111	5.21	92.2
Hymecromone	$C_{10}H_8O_3$	176.0484	5	92.1
Aldimorph	$C_{18}H_{37}NO$	283.2884	5.68	91.9
Santonin	$C_{15}H_{18}O_3$	246.127	4.17	91.9
Tritosulfuron	$C_{13}H_9F_6N_5O_4S$	445.0293	1.9	91.9
Atrazine-desethyl (Desethylatrazine)	$C_6H_{10}ClN_5$	187.0621	3.84	91.1
Metolachlor OXA (Metolachlor OA)	$C_{15}H_{21}NO_4$	279.1479	3.12	90.8
Bendiocarb/ Dioxacarb	$C_{11}H_{13}NO_4$	223.0844	3.32	89.8
Ethofumesate	$C_{13}H_{18}O_5S$	286.086	4.28	89.8
Heliotrine	$C_{16}H_{27}NO_5$	313.1895	3.29	89.8
Oxibendazole	$C_{12}H_{15}N_3O_3$	249.1127	5.2	89.5
Cinerine I (Cinerin I)	$C_{20}H_{28}O_3$	316.2043	4.49	89.2
Thionazine (Zinophos)	$C_8H_{13}N_2O_3PS$	248.038	1.14	89
Fluazifop-P-butyl	$C_{19}H_{20}F_3NO_4$	383.1371	3.74	88.3
Piperazine	$C_4H_{10}N_2$	86.0844	7.84	88
TEA / Triethylamine	$C_6H_{15}N$	101.1203	1.26	88
Acetochlor OXA (Acetochlor OA)	$C_{14}H_{19}NO_4$	265.1311	1.85	87.8
Carvacrol (Isopropyl cresol)	$C_{10}H_{14}O$	150.1045	3.02	87.7
Dicyclopentadiene	$C_{10}H_{12}$	132.0939	2.71	87.7
Furaltadone	$C_{13}H_{16}N_4O_6$	324.1065	1.6	87.7
Arecoline	$C_8H_{13}NO_2$	155.0945	2.85	87.6
Cycluron	$C_{11}H_{22}N_2O$	198.1734	4.52	87.6
OMPA / Schradan	$C_8H_{24}N_4O_3P_2$	286.1319	2.82	87.6
Xylylcarb	$C_{10}H_{13}NO_2$	179.0945	3.34	87.6

Carbofuranphenol-3-keto	$C_{10}H_{10}O_3$	178.0631	4.06	87.4
Theobromine	$C_7H_8N_4O_2$	180.065	3.01	87.3
ICIA0858	$C_{10}H_{12}N_2O_2$	192.0888	1.79	87.2
Metyridine	$C_8H_{11}NO$	137.0838	1.38	87.2
Phenylacrylic acid (Cinnamic acid)	$C_9H_8O_2$	148.0527	2.66	87.2
Proximpham	$C_{10}H_{12}N_2O_2$	192.0888	1.79	87.2
Carbofuran	$C_{12}H_{15}NO_3$	221.1061	2.89	87
Fludioxonil	$C_{12}H_6F_2N_2O_2$	248.0379	1.14	87
Butopyronoxyl	$C_{12}H_{18}O_4$	226.1205	3.51	86.8
Phenol	C_6H_6O	94.0416	1.95	86.7
4-Methylphenol (p-Cresol)	C_7H_8O	108.0575	3.24	86.6
Ethyl N-acetyl-N-butyl-Œ≤-alaninate	$C_{11}H_{21}NO_3$	215.1523	3.87	86.6
Alantolactone	$C_{15}H_{20}O_2$	232.1462	3.92	86.4
Cycloheximide	$C_{15}H_{23}NO_4$	281.1628	4.17	86.4
Metominostrobin, E- (SSF-126)	$C_{16}H_{16}N_2O_3$	284.1163	2.81	86.4
BPA / Bisphenol A	$C_{15}H_{16}O_2$	228.1153	4.17	86.3
TBP / Tributylphosphate	$C_{12}H_{27}O_4P$	266.1642	5.6	86.3
Benzadox	$C_9H_9NO_4$	195.0532	3.49	86.2
Gemfibrozil	$C_{15}H_{22}O_3$	250.1564	3.87	86.2
Diethofencarb	$C_{14}H_{21}NO_4$	267.1468	2.73	85.9
Eugenol	$C_{10}H_{12}O_2$	164.084	3.29	85.9
Isoxadifen-ethyl (AE F122006)	$C_{18}H_{17}NO_3$	295.1209	3.91	85.9
TPPA / Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.0707	5.55	85.7
Anabasine	$C_{10}H_{14}N_2$	162.1158	2.81	85.6
Coumafuryl	$C_{17}H_{14}O_5$	298.085	4.46	85.6
Desmedipham	$C_{16}H_{16}N_2O_4$	300.1115	2.89	85.6
Methfuroxam	$C_{14}H_{15}NO_2$	229.109	2.9	85.6
Nicotine	$C_{10}H_{14}N_2$	162.1158	2.81	85.6
Terbutaline	$C_{12}H_{19}NO_3$	225.1373	3.81	85.6
Salbutamol (Albuterol)	$C_{13}H_{21}NO_3$	239.1524	4.38	85.4
Aspidinol	$C_{12}H_{16}O_4$	224.1037	3.32	85.3
Avobenzone (BM-DBM)	$C_{20}H_{22}O_3$	310.1567	4.53	85.3
Bucarpolate	$C_{16}H_{22}O_6$	310.1418	3.29	85.3
Butacarb	$C_{16}H_{25}NO_2$	263.1884	4.68	85.3
Trinexapac	$C_{11}H_{12}O_5$	224.0683	3.23	85.3
Pymetrozine	$C_{10}H_{11}N_5O$	217.095	1.93	85.2
Fenoxycarb	$C_{17}H_{19}NO_4$	301.1324	4.37	85.1
Dimetan	$C_{11}H_{17}NO_3$	211.1202	3.51	85
Pyriminil (Pyrinuron)	$C_{13}H_{12}N_4O_3$	272.09	6.08	84.9

Citronellal hydrate	$C_{10}H_{20}O_2$	172.1464	4.44	84.8
Indolepropionic acid	$C_{11}H_{11}NO_2$	189.0787	3.18	84.8
Norethynodrel	$C_{20}H_{26}O_2$	298.1935	4.49	84.7
Dimidazon	$C_{12}H_{12}N_2O_3$	232.0856	2.82	84.6
BBP / Benzyl butyl phthalate (Butylbenzylphthalate)	$C_{19}H_{20}O_4$	312.1361	5.8	84.5
Metobenzuron	$C_{22}H_{28}N_2O_5$	400.1992	3.26	84.5
Clotrimazole	$C_{22}H_{17}ClN_2$	344.109	3.44	84.4
Atraton	$C_9H_{17}N_5O$	211.1419	2.78	84.2
Metolachlor CGA 357704	$C_{14}H_{17}NO_5$	279.1104	2.8	84.2
Propazine-hydroxy	$C_9H_{17}N_5O$	211.1419	2.78	84.2
Isocarbamide	$C_8H_{15}N_3O_2$	185.1157	1.36	84.1
Furmecyclox	$C_{14}H_{21}NO_3$	251.151	2.71	84
Lobendazole	$C_{10}H_{11}N_3O_2$	205.0865	2	84
Picaridin (Bayrepel) (Icaridin)	$C_{12}H_{23}NO_3$	229.1679	4.09	83.9
Diflufenzopyr (BAS 65400H)	$C_{15}H_{12}F_2N_4O_3$	334.0874	1.71	83.8
Triazophos oxon	$C_{12}H_{16}N_3O_4P$	297.0888	2.77	83.2
Benfuresate	$C_{12}H_{16}O_4S$	256.0747	5.1	82.9
Carbaryl	$C_{12}H_{11}NO_2$	201.0781	1.86	82.9
Fenfuram	$C_{12}H_{11}NO_2$	201.078	1.86	82.9
3-Hydroxycarbofuran	$C_{12}H_{15}NO_4$	237.0998	1.96	82.7
DAS / Diacetoxyscirpenol	$C_{19}H_{26}O_7$	366.1694	2.89	82.7
Isolan	$C_{10}H_{17}N_3O_2$	211.1332	7.84	82.7
Diprogulic acid (Dikegulac acid)	$C_{12}H_{18}O_7$	274.1032	2.11	82.5
Nitrothal-isopropyl	$C_{14}H_{17}NO_6$	295.1055	2.88	82.4
DNOP / Dioctyl phthalate	$C_{24}H_{38}O_4$	390.2769	6.19	81.9
Kasugamycin	$C_{14}H_{25}N_3O_9$	379.1575	4.19	81.7
N-Methyl-N-1-naphthyl acetamide	$C_{13}H_{13}NO$	199.0991	2.85	81.6
Prohydrojasmon	$C_{15}H_{26}O_3$	254.1879	3.26	81.5
Fenazox (Azoxybenzene)	$C_{12}H_{10}N_2O$	198.0803	2.85	81.4
Trifopsime	$C_{19}H_{18}F_3NO_4$	381.1205	3.69	81.3
Difenopenten	$C_{18}H_{15}F_3O_4$	352.0936	2.84	80.8
Dimantine (Dymanthine)	$C_{20}H_{43}N$	297.3395	4.43	80.6
Bilanafos	$C_{11}H_{22}N_3O_6P$	323.1232	2.19	80.1
Fluenethyl	$C_{16}H_{15}FO_2$	258.1064	1.41	80

Table 3 9 Tentative compounds detected in urban honey using the NTA

3.5. Conclusion

A rapid and validated method using direct injection HPLC-QTOF-MS was developed for the targeted analysis of 21 pesticides in urban honey. This method was able to detect pesticides at much lower levels than the regulatory limits, with good recoveries, linearity, and repeatability. The method was applied to 125 urban honey samples from Montreal, where a targeted screening using 79 analytes was first conducted. Although no pesticides were found above the MDL in the targeted analysis, a non-targeted approach was also used and revealed the presence of 111 tentative compounds with scores above 80%. While the study demonstrated the effectiveness of the method in detecting trace levels of pesticides in honey, the non-targeted approach highlights the need for further research to assess the presence of unknown compounds in city honey.

Furthermore, the study also evaluated the physicochemical properties of urban honey from Quebec, including pH, EC, moisture, and color. The physicochemical properties of urban honey were within acceptable ranges and similar to rural honey. However, the higher EC values in urban honey highlight the need for additional analytical methods, such as chemical fingerprinting, to accurately differentiate between urban and rural honey samples, as physicochemical properties alone are insufficient for this classification.

In conclusion, the study provides two pivotal findings. Firstly, the developed method for detecting pesticides in honey is proven to be efficient, exhibiting both robustness and sensitivity. Secondly, the study offers reassurance that the levels of pesticides in urban honey are not of concern. Furthermore, the non-targeted approach's revelation of unknown compounds necessitates additional investigation, underscoring the imperative of sustained monitoring and research to safeguard the quality and safety of urban honey for human consumption.

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CONNECTING PARAGRAPH

Chapter 3 of the study focused on the non-targeted analysis of pesticides in urban honey samples using direct injection HPLC-QTOF-MS. This approach allowed for the detection of unknown compounds that require further investigation to determine their potential impact on human health and the environment. In Chapter 4, the study expands its scope to include the analysis of pesticides in urban honey and passive air samplers for monitoring pesticides in urban air. The aim of Chapter 4 is to assess the potential sources and pathways of pesticide contamination in urban environments. By analyzing both the honey and the air, the study can provide a more comprehensive understanding of pesticide exposure in urban areas. Together, these chapters offer valuable insights into the presence or absence of pesticides in urban environments, highlighting the need for more extensive monitoring and evaluation to protect human health and the environment.

Chapter 4: Analysis of passive air samplers and urban honey for pesticides in urban environment

4.1. Abstract

The objective of this investigation was to evaluate urban honey's potential as a complementary tool for assessing airborne pesticides, comparing it with outcomes from an artificial XAD-resin-based passive air sampler (XAD-PAS). A total of 118 urban honey samples were gathered from the city of Montreal in Quebec, Canada, and 40 sites (residentials and parks) across Montreal were selected to deploy the XAD-PAS over a period of three months in the summer of 2021. A method that combines direct injection with high-performance liquid chromatography and quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS), was utilized as a screening technique to identify pesticides in both matrices obtained from urban areas. This approach known as non-targeted screening, does not target specific pesticides but aims to detect any present in the sample. DEET, the widely used insect repellent, was the only compound out of the 29 molecular features detected, whose identity was confirmed through MS/MS fragmentation, comparison with the Agilent PCDL database, and the use of an analytical standard. The highest concentration of DEET was found in a public park in Maisonneuve. DEET peak areas in residential areas and parks were then compared using statistical analysis. The results revealed no significant differences in DEET levels between these two locations. This emphasizes consistent DEET presence and suggests distribution equilibrium, enriching our understanding of pesticide dynamics in urban spaces. Finally, an evaluation of contamination in honey and air matrices was performed to assess their suitability for measuring pesticides. This analysis revealed the absence of DEET in urban honey, while suggesting its potential for tentative pesticide identification. The integration of urban honey and XAD-PAS offered comprehensive insights into pesticide contamination. In conclusion, this research presents an innovative utilization of non-targeted analysis through XAD-PAS to assess urban air quality and investigate how urban honey can serve as a supplementary method to evaluate airborne pesticides.

4.2. Introduction

Pesticides, as a type of semi-volatile organic contaminant, have been identified as one of the most prevalent contaminants in the environment (Lévy et al., 2018). Pesticides can be released into the atmosphere through various mechanisms, including evaporation, volatilization, or spraying using equipment (Cabrerizo et al., 2011; Climent et al., 2019). When sprayed, only 70% of the pesticides reach their target, leaving the remaining 30% in the air, as observed in studies (Fuhrimann et al., 2020; Van Den Berg et al., 1999). Furthermore, depending on their lifespan, pesticides can be transported over long distances from their application sites (Degrendele et al., 2016; Zhang et al., 2013), dispersing through the air and reaching various regions, including urban and remote areas (Shen et al., 2005). Several studies from different regions worldwide, including Europe (Schummer et al., 2012), Africa (Fuhrimann et al., 2020; Isogai et al., 2018), Asia (Shunthirasingham et al., 2010), North America (Daly et al., 2007), and even the poles (Bengtson Nash et al., 2017), have detected airborne pesticides, highlighting the global prevalence of this issue (Martin et al., 2022).

Two main sampling techniques have been commonly employed to assess the presence of pesticides in the air, passive air sampling (PAS) and active air sampling (AAS). In these processes, air sampling is either active or passive and allows for an accurate qualitative and quantitative assessment of air quality (Wang et al., 2016). AAS has been the traditional method for sampling pesticides for many years (Al-Alam et al., 2021). Using a pump with a well-defined flow rate, pollutants are trapped by a filter (to capture compounds bound to particles) and/or an adsorbent bed (to capture compounds in the gas phase) (Al-Alam et al., 2019; Lévy et al., 2020; Tuduri et al., 2012). Although AAS allows the precise measurement of pesticide fluctuations daily, it is

limited by several drawbacks, including expensive air sampling pumps, frequent calibrations, and the need for power sources. Thus, passive air samplers (PAS) were favored in many environmental studies to overcome these problems by allowing an integrated measurement of pesticide concentrations over a longer sampling period (Gamboa et al., 2020). Passive sampling, defined by Górecki and Namienik in 2002, is a sampling method based on the free movement of analyte molecules from the sampled medium to the collection medium (Górecki & Namieśnik, 2002).

Honeybees, also known as *Apis mellifera*, play a critical role in pollinating agricultural crops and native species, making them essential to the production of commercial honey and beeswax. Their daily foraging activities cover a vast area of approximately 7 km^2 near their hive in search of nectar, water, and pollen from flowers (Rissato et al., 2006). However, during their foraging, honeybees are exposed to various microorganisms, chemical products, and particles, including pesticides, which are harmful and can even end up in the honey they produce (Devillers & Pham-Delegue, 2002). As bio-indicators, honeybees' foraging activities and products provide valuable insights into the health of the environment. They interact with almost all environmental sectors, including soil, vegetation, water, and air, and are sensitive to various biological, chemical, and physical factors (Celli & Maccagnani, 2003; Fernández et al., 2002; Kevan, 1999). Therefore, it is crucial to protect honeybees from exposure to harmful contaminants and pesticides to ensure the production of high-quality honey. Honey, in particular, is an ideal bioindicator since worker bees collect nectar and pollen from flowers, interact with all aspects of the environment, and can carry small particles of air containing pesticides back to the hive (Smith et al., 2019). Consequently, honey can become contaminated with various substances, including pesticides present in the air, which can then become incorporated into the honey (Smith et al., 2019). Thus, honey serves as a

record of pesticide exposure in the environment where bees collect nectar. By monitoring the quality of honey, it is possible to assess the environmental stress levels and take appropriate measures to protect honeybees from harmful contaminants and pesticides.

PAS is generally more expensive than environmental bioindicators, especially in emerging economies, limiting the number of samplers deployed simultaneously at various sampling sites (Silva-Barni et al., 2019). Using environmental tools and PAS simultaneously is key to comparing and validating their performance monitoring atmospheric pollutants. In previous studies, SVOCs accumulation in ginkgo leaves and active air samples were compared (Murakami et al., 2012), pine needles and Hi-Vol (Klánová et al., 2009), PUF and Tillandsia epiphytes (Wannaz et al., 2013), XAD-PAS and lichens and conifer needles (Schrlau et al., 2011), XAD-PAS and lichens (Daly et al., 2007), XAD-PAS and T. bergeri epiphyte (Silva-Barni et al., 2019), and PUF and honeybees (Ayyildiz et al., 2019). The results of these comparisons varied depending on the type of sampler and bioindicators used, implying that each media must be compared individually to different types of artificial sampler (Silva-Barni et al., 2019). Through the strategic combination of PAS and honey samples, a heightened level of accuracy in tracking pesticides within the surrounding atmosphere can be attained. Furthermore, this collaborative approach enables a more thorough assessment of honey's suitability as a bioindicator for detecting airborne pesticides.

The indiscriminate application of pesticides in urban environments like lawns, gardens, and parks results in many accidents and exposures worldwide. A typical urban homeowner uses ten times more chemical pesticides per acre than a typical farmer (US FWS, 2000). In addition, SVOCs are extensively transported from towns and cities to their surroundings and the food web, causing significant environmental impacts on a regional scale (Hodge & Diamond, 2009). Thus, a better

understanding of SVOC sources, quality, and quantity in the urban area is necessary to reduce their emissions and concentrations in the environment. Monitoring pesticides in cities is important for protecting human health, the environment, and pollinators, and for understanding trends in pesticide use and the effectiveness of efforts to reduce or eliminate their use. Passive air samplers (PASs) can monitor the presence of pesticides in the air around beehives, which can help identify potential sources of contamination and assess the potential risks to bees and the quality of the honey they produce. In 2018, Lévy et al. monitored pesticide concentrations and variations in the ambient air at three different sites (rural, urban and suburban) in France for 4 years using XAD-resin PASs. The results showed different patterns of pollutant accumulation between the rural and urban sites, with different proportions of pesticides observed depending on whether they were applied for domestic use or released into the environment and decomposed by various environmental and meteorological conditions (Lévy et al., 2018). The objective of this study was to assess the reciprocal benefits and synergies between pesticide measurement in honey and air, offering a multifaceted perspective on pesticide exposure within urban settings, using Montreal as a case study, as an effective indicator of air pollution levels measured through passive air sampling. In order to achieve this objective, a non-targeted approach utilizing LC-MS will be employed.

4.3. Methodology

4.3.1. Passive air samplers

4.3.1.1. Chemicals and reagents

A LC mix recovery standard was prepared by mixing, tetrabromobisphenol A-d4 (TBBPA-d4), azoxystrobin-d4, bifenthrin-d5, diuron-d6, atrazine-d5, clothianidin-d3, imidacloprid-d4, metolachlor-d6, aminomethane phosphoric aicd-$^{13}C_{12}$,-d2 (AMPA-$^{13}C_{12}$, N-d2), glyphosate-d2,

triclosan-d3, bisphenol A-$^{13}C_{12}$, bisphenol S-$^{13}C_{12}$, bisphenol AF-$^{13}C_{12}$, (BPAF-$^{13}C_{12}$, diethyl phtalate-d14 (DEP-d14), dibutyl phosphate-d14 (DiBP-d4), n-butyl benzyl phthalate (BBzP-d4), dicyclohexyl phthalate-d4 (DCHP-d4), di-(2-ethylhexyl) phthalate-d38 (DEHP-d38) and di-n-octyl phthalate-d4 (DnOP-d4). Labeled standards were brought from Agilent Technologies (Santa Clara, CA, USA), Cambridge Isotope Laboratories (Saint-Laurent, QC, Canada), C/D/N Isotopes (Pointe-Claire, QC, Canada), Sigma-Aldrich (Burlington, MA, United States), and Toronto Research Chemicals (North York, ON, Canada) (**Table 4** ***1***). An LC mix of injection internal standard was prepared by mixing carbamazepine-d10, gemfibrozil-d6, caffeine-d3, and terephthalic acid-d4. HPLC-grade solvents, including acetone, hexane, isooctane, and methanol, were purchased from Fisher Scientific (Hampton, NH, United States). Ottawa Sand was purchased from Fisher Chemical (Pittsburgh, PA, USA).

Compound	**Vendor**
TBBPA-d4	C/D/N Isotope
AMPA-$^{13}C_{15}N$-d2	Agilent Technologies
Atrazine-d5	Cambridge Isotope Laboratories
Azoxystrobin-d4	Sigma-Aldrich
BBzP-d4	C/D/N Isotope
Bentazone-d7	Toronto Research Chemicals
Bifenthrin-d5	C/D/N Isotopes
Bisphenol A -$^{13}C_{12}$	Cambridge Isotope Laboratories
Bisphenol S-$^{13}C_{12}$	Toronto Research Chemicals
BPAF-$^{13}C_{12}$	Toronto Research Chemicals
Caffeine-d3	Cambridge Isotope Laboratories
Carbamazepine-d10	C/D/N Isotope
Clothianidin-d3	C/D/N Isotopes
DEHP-d38	Toronto Research Chemicals
DEP-d14	C/D/N Isotope
DiBP-d4	C/D/N Isotope
Dicyclohexyl phthalate-d4	C/D/N Isotope
Diuron-d6	Cambridge Isotope Laboratories
DnOP-d4	C/D/N Isotope
Gemfibrozil-d6	C/D/N Isotopes

Glyphosate-d2	C/D/N Isotopes
Imidacloprid-d4	Sigma-Aldrich
Metolachlor-d6	C/D/N Isotopes
Terephthalic acid-d4	C/D/N Isotopes
Triclosan-d3	C/D/N Isotope

Table 4 1 List of the isotopically labeled standards and their vendors.

4.3.1.2. Sampler Design & Deployment

PASs consist of a bottom part of a stainless-steel shelter in which two cylindrical, stainless-steel mesh cylinders are suspended to a stainless-steel lid. The mesh cylinder reduces potential contamination and adsorptive wall loss. As a sampling medium, the cylinders were filled with the sorbent resin XAD-2 resin, which is extensively used for routine monitoring of the POPs (Wania et al., 2003). XAD-2 is a hydrophobic sorbent, and most POPs are halogenated hydrocarbons without hydrophilic substituents. Therefore, water is unlikely to influence the sorption of POPs to XAD-2, and humidity is unlikely to affect the uptake of POPs to the XAD-2 (Wania et al., 2003). As for the PAS design, the shelter's bottom opening is intended to reduce the impact of wind speed and shield the sampling medium from precipitation and large aerosol particles subject to gravitational settling. The stainless-steel lid and bottom parts fit snugly into each other and are robust enough to withstand severe weathers condition. A carabine hook is attached to the inside of the lid and allows the cylinder to hang. As a result, the operator does not have to touch the resin-filled container, and the shelter can be opened and closed repeatedly. Spokes fitted into the cover prevent accidental unhooking of the cylinder. In the shelter, air exchanges occur through a bottom opening and several small holes in the top. A mesh grid is inserted into the bottom opening to keep larger animals away from the sampling cylinder while maintaining an adequate airflow (Wania et al., 2003).

As the rates of PAS uptake are dependent on variables such as wind speed, humidity, temperature, and other environmental factors (Grosse & McKernan, 2014), samples were deployed in the summertime at a time when these factors are primarily stable and pollutants such as pesticides, are likely to be present at their peak. The (PAS)s were deployed during summer, from mid-July to the first week of October 2021 (81 days). In the field, the sampling cylinder is attached to an existing structure or a pole at around 1-1.5m above the ground. As for transportation, the sampling cylinder is enclosed in a Teflon tube with a double-lid design to prevent rainwater from entering (Wania et al., 2003). The University of Toronto (Prof. F. Wania) provided the XAD-resin. An extensive explanation of sampler design and resin cleaning has been described in the literature (Wania et al., 2003).

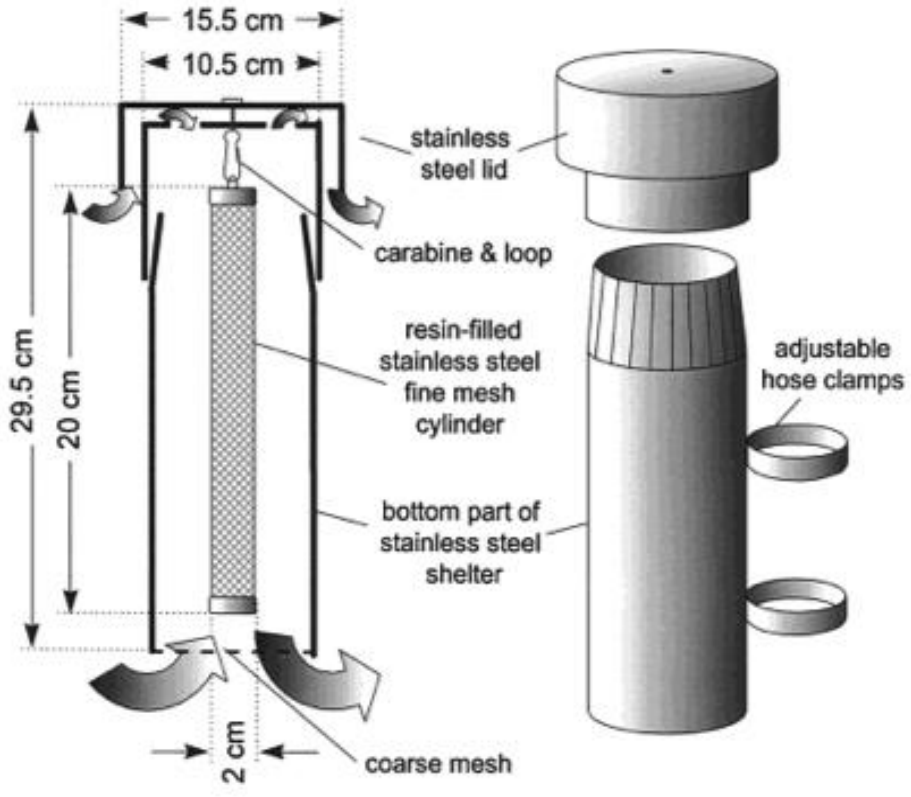

Figure 4 1 XAD-based passive air sampler's design and dimension. Retrieved from (Wania et al., 2003

4.3.1.3. Field Sampling Site of PASs

Located in the southwest of the province of Quebec, Montreal is the main island of the Hochelaga Archipelago at the confluence of the Saint Lawrence and Ottawa rivers. The air sampling campaign was conducted on the Island of Montreal, 364.74 $km^{2,}$. Around 1.7 million people live on this island, with a population density of 4,833/km^2 (Statistics Canada, 2021). Forty PASs were deployed all over Montreal, 25 samples were deployed in residential and 15 in public locations. PASs were not deployed with a specific spatial distribution due to the dependency on resident approvals and public park permissions. However, a significant number of the air samplers were positioned in close proximity to the beehives from which the honey samples were collected.

Upon collection, the mesh cylinders were transferred to metal shipping tubes closed with a plastic cap and sealed with Teflon rubber for transport. The samplers were frozen at -80°C until analysis.

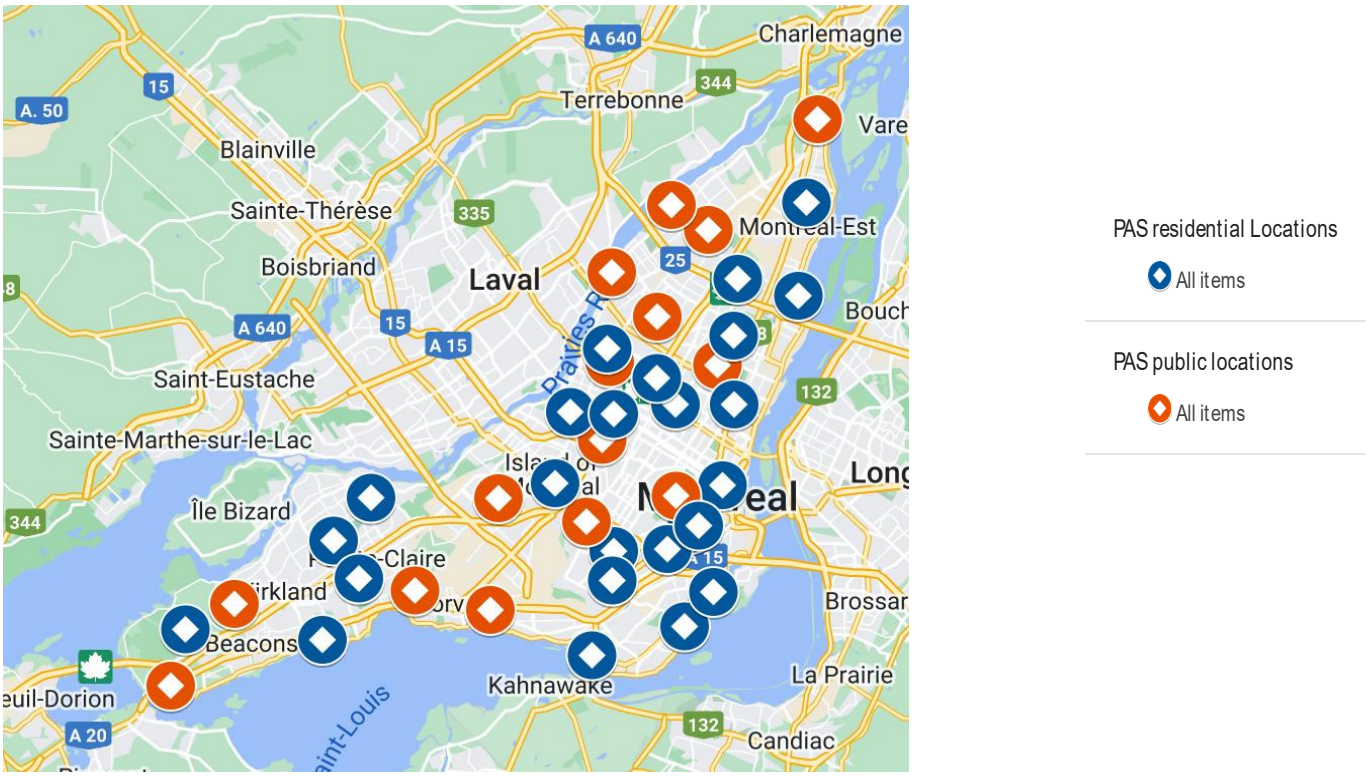

Figure 4 2 Map of passive air samplers' locations in Montreal

4.3.1.4. QA/QC

One day before the extraction, the glass containers underwent a thorough cleaning process followed by exposure to an oven temperature of 325°C for 4 hours, and they were also rinsed with hexane and acetone both before and after each use. Six XAD-2 procedural blanks, three field blanks, two field replicates, and forty samples were extracted and analyzed. A standard quality assessment procedure was followed in this study. Procedural blanks were extracted and analyzed with the exposed samples to determine whether background contamination was initially in the XAD-2 resin and possibly introduced during extraction and chemical cleaning. Moreover, two field duplicates were deployed to evaluate how field conditions affect precision. Additionally, three XAD-PAS field blanks involved taking the passive sampler to the designated sampling locations and leaving it there to be exposed to the surrounding air during both the installation and collection stages, in order to assess any potential contamination. In this study, the blanks served as a means of assessing possible contamination caused by handling, shipping, and storage.

4.3.1.5. Extraction of the contaminants from PASs

Before extraction, samples were spiked with 50 μL of LC mix recovery standard. The XAD-2 filled sampling cylinders were then extracted using the Dionex™ ASE™ 350 Accelerated Solvent Extractor (Thermo Fisher Scientific, Hampton, NH, United States) using a mixture of acetone/hexane (50/50, v/v), both pesticides grade (Fisher Chemical, Hampton, NH, United States). The ASE conditions and methods used in this study were based on those described in references (Zhang et al., 2017; Zhang et al., 2012), with modifications made to suit the specific experimental requirements. The extractions were conducted for three cycles of 6 minutes at 1500 psi and 75°C. The extract was then concentrated using a rotary evaporator (Büchi, Lukens Drive,

New Castle, USA) to 1 mL, then transferred to a baked glass test tube and rinsed three times with a whole baked pipette mixture of acetone and hexane (50/50, v/v). The sample was further reduced to approximately 0.5 mL under a stream of nitrogen. Subsequently, the 0.5 mL were transferred to the LC vial and rinsed with 0.5 ml of the mixture of acetone and hexane (50/50, v/v) twice and completely dried using N_2 blow. Finally, 475 µL of methanol was used to reconstitute the sample, and 25 µL of LC Mix injection internal standard, which was used as injection standard for LC analysis, was added. Samples were vortexed gently for 1 minute before being analyzed using the LC-MS.

4.3.1.6. Instrumental analysis

For the LC-MS analysis, the samples were also filtered, using 0.22 µm PTFE syringe filters and acidified using methanol (LC-MS grade). Agilent 1290 Infinity II LC system coupled to the 6545 Q-TOF-MS was applied for data collection (Agilent Technologies, Santa Clara, USA). The LC separation was performed on a Poroshell 120 EC-C18 analytical column (Agilent Technologies; 2.7 µm × 3 mm × 100 mm) connected with a Poroshell120 EC-C18 guard column (Agilent Technologies; 2.7 µm × 3 mm × 5 mm). The mobile phase A was HPLC water. The mobile phase B was an acetonitrile/methanol mixture (50:50 v/v), with ammonium acetate (5 mM) added to both mobile phase A and B. Samples were kept at 4°C in the multi-sampler compartment. HPLC parameters for both ion modes were the flow rate of 0.3 $mL.min^{-1}$, the injection volume of 4 µL, column temperature of 30°C. The elution gradient was: 5% B (0 to 0.5 min), linear increase to 100% B (0.5 to 4 min), 100% B (4-8 min), decrease to 5% B (8 to 8.01 min) and finally 5% B (8.01 to 9 min) with 2 min post-column run. MS conditions were: the drying gas temperature at 175°C, drying gas flow rate at 11 L/min, sheath gas temperature at 375°C, sheath gas flow rate at 12 L/min, nebulizer pressure at 30 psi, the capillary voltage at 4000 V, the fragmentor voltage at

125 V, the skimmer voltage at 45 V and the nozzle voltage at 1000 V. Full scan MS data were recorded at mass-to-charge ratio (m/z) range from 70 to 1700 with a scan rate of 2 spectra/s, and were collected using both centroid and profile modes. Two reference ions (*m/z* at 121.0508 and 922.0098 for ESI+, 112.9856 and 1033.9881 for ESI-) were used in each ion mode for automatic mass recalibration during data acquisition.

4.3.1.7. Data treatment

Data treatment was conducted in the "Batch Targeted Feature Extraction" mode (RT tolerance ± 0.15 min, mass tolerance ± 20 ppm, absolute height threshold ≥ 10000 counts, score ≥ 80) for full scan MS data and the resulting MS information was screened with the library Pesticides PCDL (Agilent Technologies).

4.3.2. Honey samples

Data from honey samples used in the previous chapter were used. Please refer to Chapter 3.

4.4. Results and discussion

4.4.1. QA/QC for air sampler analysis

The apparent recoveries of mass-labeled surrogates were satisfactory in the ESI+ mode. In the ESI- mode, the recoveries of Triclosan (110±32%) and TBBPA-d4 (30±14%) were found to be satisfactory, while the mass-labeled bisphenols were not recovered due to reasons that remain unclear. Nevertheless, a test with native bisphenol revealed that the instrument conditions were adequate for detecting bisphenols, suggesting that the loss occurred during the extraction process. The internal standards for the instrument (2 in ESI+ and 2 in ESI-) produced good signals in all

samples, indicating that the instrument analysis was stable throughout the runs. Overall, the findings of this study indicate that the extraction method utilized for resin was effective in extracting the majority of compounds, except for certain compound families, such as bisphenols, which exhibited lower extraction efficiency.

Spiked ISTD before extraction	**Formula**	**m/z**	**Ion mode**	**RT**	**Apparent recovery%**
Atrazine-d5	$C_8H_9D_5ClN_5$	221.1324	ESI+	5.451	65±19%
Azoxystrobin-d4	$C_{22}H_{13}D_4N_3O_5$	408.1498	ESI+	5.576	80±23%
Benzyl-n-butyl phthalate-d4	$C_{19}H_{16}D_4O_4$	317.1691	ESI+	6.114	50±11%
Clothianidin-d3	$C_6H_5D3ClN_5O_2S$	253.0348	ESI+	4.591	73±21%
DEHP-d38	$C_{24}D_{38}O_4$	429.5233	ESI+	7.538	89±59%
DiBP-d4	$C_{16}H_{18}D_4O_4$	283.1848	ESI+	6.131	43±23%
DCHP-d4	$C_{20}H_{22}D_4O_4$	335.2161	ESI+	6.586	74±24%
Diuron-d6	$C_9H_4D_6Cl_2N_2O$	239.0625	ESI+	5.609	67±20%
DnOP-d4	$C_{24}H_{34}D_4O_4$	395.31	ESI+	7.935	77±24%
Imidacloprid-d4	$C_9H_6D_4ClN_5O_2$	260.0847	ESI+	4.5	83±24%
Metolachlor-d6	$C_{15}H_{16}D_6ClNO_2$	290.1788	ESI+	5.932	76±22%
Bisphenol S-13C12	$[13C]12H_{10}O_4S$	261.0624	ESI-	4.722	Not detected
Bisphenol A-13C12	$C_3[13C]12H_{16}O_2$	239.1474	ESI-	5.343	Not detected
BPAF-13C12	$C_3[13C]12H_{10}F_6O_2$	347.0909	ESI-	5.55	Not detected
TBBPA-d4	$C_{15}H_8D_4Br_4O_2$	546.7702	ESI-	6.088	30±14%
Triclosan-d3	$C_{12}H_4D_3Cl_3O_2$	289.9622	ESI-	6.146	110±32%

Instrument ISTD	**Formula**	**m/z**	**ion mode**	**RT**	**matrix effect**
Carbamazepine-d10	$C_{15}H_2D_{10}N_2O$	247.1655	ESI+	5.32	135±46%
Caffeine-d3	$C_8H_7D_3N_4O_2$	198.107	ESI+	4.277	128±46%
Terephthalic acid-d4	$C_8H_2D_4O_4$	169.0439	ESI-	1.406	166±34%
Gemfibrozil-d6	$C_{15}H_{16}D_6O_3$	255.1867	ESI-	5.985	378±60%

Table 4 2 Isotopically mass-labeled standards and their respective recoveries

4.4.2. Principal component analysis

To ensure data quality and identify any instrumental artifacts, an unsupervised method, such as principal component analysis (PCA), was employed. The dataset comprised 14 samples, including 2 solvent blanks, 3 procedural blanks, and 3 field blanks. By examining the positions and groupings of the various blank samples in the PCA plot, the analysis's reproducibility and quality were determined. Both positive and negative modes were utilized for molecular entity analysis. Notably, the results of the PCA (**Figure 4** ***3***) exhibited distinct groupings of the solvent blanks, procedural blank, and field blanks, which were separate from the real samples. This distinction suggests significant differences between the controls and the actual samples.

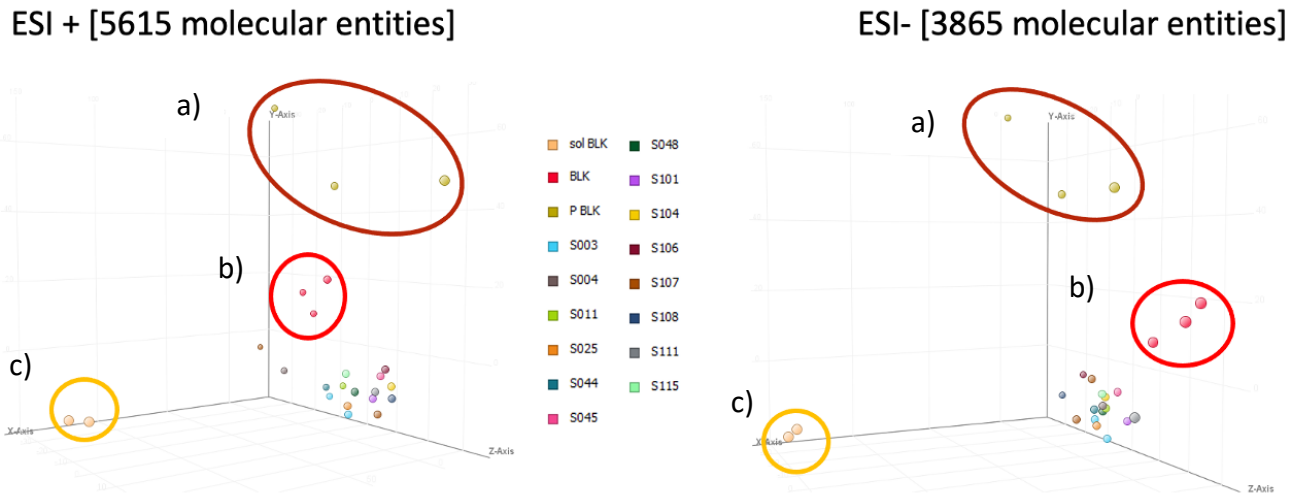

Figure 4 3 Principal component analysis (first three components) for LC-MS data in ESI+ and ESI- ionization modes, showing a proper grouping of a) procedural blanks, b) field blanks and c) solvent blanks in both modes

4.4.3. LC-MS analysis

In the full MS data, molecular features were tentatively identified, and only those with signals significantly higher than that in the blanks were selected for targeted MS/MS fragment comparison

with the MS/MS library. The tentatively identified molecular features were confirmed using authentic standards based on retention time and targeted MS/MS fragments.

A total of 29 molecular features corresponding to contaminants with matched MS/MS information or high SIRIUS scores were successfully detected, of which 5 were identified as pesticides. However, due to cost constraints, limited availability of analytical standards, and time limitations, only one insect repellent, DEET (N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide), was confirmed at level 1 according to the Shymanski scale (Hollender et al., 2017). However, DEET was also the only detected pesticide in all of the blanks. To address this concern, a criterion was applied where only peak areas with intensities exceeding 100K in at least one sample were selected. This threshold was deemed reasonable to ensure optimal peak shape, signal-to-noise ratio (S/N), and to provide sufficient intensity for subsequent MS/MS analysis. A similar concern was raised by Aerts et al. who utilized silicon wristbands as passive air samplers, where they detected DEET in all blanks (Aerts et al., 2018). DEET is one of the commonly found substances in the environment, along with other chemicals like nanomaterials, pesticides, pharmaceuticals, industrial compounds, and personal care products (Stuart et al., 2012). During testing, DEET, the most widely used and effective insect repellent (Koren et al., 2003), was detected in 28 out of the 40 air passive samplers. Initially, the identity of DEET was verified by subjecting it to MS/MS fragmentation and comparing the resulting data with the Agilent PCDL database. To further corroborate the identification of DEET, an analytical standard of DEET was obtained from Sigma Aldrich and employed to validate its identity. The highest concentration of DEET was found in a public park in Maisonneuve, surpassing other locations in Montreal, including Verdun, which had the second-highest concentration. Variations in DEET concentrations can be influenced by multiple factors, such as increased usage of insect repellent in parks during peak mosquito seasons

and rainfall (Marques dos Santos et al., 2019). Several studies have assessed DEET in the air using PAS and consistently detected its presence. Wise et al. (2021) empl(Foyed silicone PAS and urine biomonitoring to detect

DEET in residential settings. Aerts et al. (2018) examined DEET in outdoor areas of residential settings, while Zaller et al. (2023) found DEET in 4 out of 15 PAS samples in Austria, with the highest concentration recorded in the city center. Additionally, DEET has been frequently found in aquatic systems, including urban wastewaters (Sandstrom et al., 2005).

Other pesticides have also been detected in urban settings using PAS. During April 2003, elevated concentrations of dacthal, a herbicide used to control weeds on turf grass, were detected in Toronto at 265 pg m^{-3}, indicating substantial urban usage during that specific time (Gouin et al., 2008). Like dacthal, increased levels of chlorpyrifos, an organophosphate insecticide commonly employed for managing insect pests in fruits and vegetables, were most prominent in Toronto during May 2003, reaching 670 pg m^{-3}(Gouin et al., 2008). This suggests extensive residential utilization, particularly in relation to home gardens.

To conclude, the widespread presence of DEET in both the blanks and actual PASs groups suggests that DEET is a ubiquitous environmental contaminant (Aerts et al., 2018). This holds true not just for water sources (Elliott & VanderMeulen, 2017; Merel & Snyder, 2016), but also for aerosols (Aerts et al., 2018; Balducci et al., 2012; Bergmann et al., 2017). However, despite its prevalence in various environments, there is still limited understanding regarding its occurrence in urban air. This study introduces novelty by being the first to utilize a PAS, specifically employing

XAD resin, for non-targeted analysis. This contribution enhances the limited understanding of urban air in relation to DEET contamination.

4.4.4. Spatial distribution of DEET, residential vs parks

Statistical analysis was conducted on the data of DEET to determine its distribution and to assess if there were significant differences between its levels in residential and park locations (**Figure 4 2**). The Kolmogorov-Smirnov test was used to check for normality of distribution. It was found that when all data were included, DEET did not follow a normal distribution (KSNormalP, P=0.026, KSLogNormalP, P=0). However, when the data from the highest location, Park Maisonneuve, were excluded, DEET was normally distributed (KSNormalP, P=0.568). Additionally, a T-test and Wilcoxon rank-sum test were performed to compare the mean and median values of DEET between residential and non-residential sites. The results indicated no significant differences (t-test, P=0.896, Wilcox, P=0.055), between the mean and median values of DEET in the two types of sites. These findings suggest that DEET is present at similar levels in both types of locations and its distribution may vary based on the presence of other factors, such as vegetation and sources of emissions. This study addresses a knowledge gap as no specific research has compared the distribution of pesticides in the air of residential areas and parks.

While numerous studies have examined pesticides in indoor air, only a few studies have investigated pesticide residues in outdoor air in residential areas. In 2016, a research project analyzed 360 dust samples collected from various outdoor surfaces at 40 houses, revealing the widespread presence of pesticides and their degradation products. Bifenthrin and permethrin, pyrethroids, were the most detected pesticides, comprising 55% of the total. Pesticide concentrations increased during summer due to recurring applications (Jiang et al., 2016). In

another project study, pesticides were observed in urban parks, specifically in earthworms sampled from soils in Beijing, China. These earthworms were found to contain residues of persistent organochlorine pesticides (OCPs), including DDTs and HCHs, with DDT concentrations ranging from 18.97 to 1.11 × 104 $ng.g^{-1}$, and HCH concentrations ranging from 0.65 to 44.78 $ng.g^{-1}$ (Li et al., 2010). Although no direct comparison between urban residential areas and parks has been conducted to assess significant differences, it is anticipated that parks may exhibit lower levels of air contaminants since trees in urban areas play a crucial role in mitigating air pollution by absorbing gaseous pollutants and effectively removing them from the atmosphere (McPherson, 1994; Nowak et al., 2006).

4.4.5. Urban honey and PASs

While the validated direct injection HPLC-QTOF-MS method did not detect DEET or targeted pesticides in the urban honey samples, the identification of tentative pesticides through a non-targeted approach suggests the potential presence of unknown compounds within it. In contrast, DEET was found in the passive air samplers, indicating possible exposure through air for humans and organisms such as bees. These findings emphasize the value of employing both methodologies to comprehensively understand pesticide presence in urban environments, including their dissemination pathways.

While some studies have explored airborne pesticides using bioindicator techniques and compared them with PASs, our study pioneers the use of urban honey as a complementary tool alongside PASs for assessing pesticides in the urban environment. Earlier research, such as Silva-Barni et al.'s (2019) examined the use of *Tillandsia bergeri* plants for monitoring pesticides in air and found a low correlation between pesticides in *T. bergeri* and XAD-PAS, which could be attributed to the

accumulation of compounds from different phases, namely particle and gas phases. Similar findings were reported by Schrlau et al. (2011) when comparing pesticide levels in XAD-PAS and lichens. In conclusion, while urban honey might not offer optimal reliability for monitoring certain pesticides such as DEET, it presents promise in identifying tentative pesticides in conjunction to PASs. Sustained monitoring and in-depth analysis of pesticide residues within urban honey and air samples remain essential to ensure the well-being and security of urban communities.

4.5. Conclusion

In conclusion, a method that combines dilution and shooting to solubilized samples coupled to HPLC-QTOF-MS was used for extracting compounds from resin. The method was found to be satisfactory, except for certain families of compounds, such as bisphenols, which were not efficiently extracted. Resin samples were analyzed using a suspect screening approach. The insect-repellent DEET was one of the tentative compounds. The identification of the molecular feature was confirmed using authentic standards based on retention time and targeted MS/MS fragments. DEET was found to be a ubiquitous environmental contaminant and was detected in all air passive samplers. Statistical analysis revealed that DEET was present at similar levels in both residential and non-residential locations, indicating that its distribution may vary based on the presence of other factors, such as vegetation and sources of emissions. No targeted pesticides were detected in the urban honey using the validated direct injection HPLC-QTOF-MS method. Overall, the findings suggest that the method is effective in detecting and identifying compounds of interest, but careful consideration must be given to the extraction process and potential sources of contamination to assess environmental exposure accurately.

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Chapter 5: General conclusions

5.1. Conclusion

In this study, a non-targeted analytical method was developed and validated for the detection of various pesticides families in urban honey. Initially, a rapid screening and quantification method was successfully developed and validated for the targeted analysis of 21 pesticide residues in honey, utilizing direct injection HPLC-QTOF-MS. Despite employing a dilute-and-shoot sample preparation approach without any further cleanup, the pesticides residues were detected at concentrations approximately 2 to 1000 times lower than the regulatory limits, with acceptable linearity, recoveries, and repeatability. Furthermore, employing direct injection was very time efficient, with a total analysis time of around 45 minutes per sample, encompassing sample preparation and instrumental runtime. This study successfully demonstrated the potential of this method to detect residues of urban honey at minimal levels without the need for specific sample preparation steps.

Following the successful validation of the non-targeted method, it was applied to analyze 125 urban honey samples collected from Montreal as a case study. In this analysis, 79 targeted pesticides, were used for screening and none were found above the MDL. Subsequently, the non-targeted approach was applied to the same samples, utilizing a comprehensive library of 1750 pesticides from Agilent Technologies, which resulted in the provisional identification of 111 compounds. However, none of the detected compounds were confirmed in this study. This study demonstrates the potential of the non-targeted method for the comprehensive screening of contaminants in complex matrices such as urban honey.

Moreover, the investigation also assessed the physicochemical characteristics of Montreal's urban honey from 2021, encompassing pH, EC, moisture content, and color. The measured values fell within acceptable ranges and were compared with those of rural honey from Quebec. The comparative analysis indicated no substantial distinctions between the two honey types, except for elevated EC values observed in urban honey. These findings imply that relying solely on physicochemical properties is inadequate to differentiate between urban and rural honey.

Finally, the feasibility of using urban honey as an additional tool to assess air pollution levels in conjunction with passive air sampling was explored. This investigation was conducted using Montreal as a case study. In this study, a method was used to extract compounds from the resin using direct injection with HPLC-QTOF-MS. The method was found to be satisfactory, but certain families of compounds, such as bisphenols, were not efficiently extracted. Using a suspect screening approach, resin samples were analyzed and tentatively identified the insect-repellent DEET, which was later confirmed using authentic standards based on retention time and targeted MS/MS fragments. DEET was a common environmental contaminant, present in 28 out of 40 passive air samplers analyzed. The levels of DEET were similar in residential and non-residential locations, suggesting that various factors influence its distribution. While signals similar to DEET were detected in all urban honey samples, they could not be confirmed as DEET in unspiked urban honey samples. Furthermore, no targeted pesticides were detected in the analysis of urban honey, but several tentative compounds were found in both urban honey and passive air samplers, requiring further confirmation to assess the suitability of urban honey as an environmental bioindicators for pesticides in the air. The findings suggest that the method used in this study is effective in detecting and identifying compounds of interest in resin. However, careful

consideration must be given to the extraction process and potential sources of contamination to assess environmental exposure accurately. While the results suggest that urban honey may not be the most reliable method for monitoring DEET, it may be useful for identifying tentative pesticides.

5.2. Future considerations

Following the conclusion of this thesis, various recommendations for future research have been recognized. These recommendations encompass:

- Expanding the scope of the non-targeted method to include a wider range of emerging trace contaminants in food would enhance our understanding of their occurrence and behavior in honey. This could involve optimizing the method to target specific classes of contaminants or incorporating advanced data analysis techniques to improve compound identification.
- Further investigation is needed to confirm the presence and identity of the tentative compounds detected using the non-targeted approach in honey and passive air samplers. This would involve using authentic standards to provide conclusive evidence.
- Implementing long-term monitoring programs for pesticides in honey would provide valuable insights into temporal trends.
- Conduct a comprehensive study encompassing urban honey samples from diverse geographical locations to enhance our understanding of urban honey safety on a broader scale.
- For passive air samplers, modifications to improve the extraction efficiency for compounds that were not effectively extracted, such as bisphenols.

General reference list

Please note that as per the Guidelines for Thesis Preparation, individual reference lists are included within each manuscript chapter (i.e., Chapters 3-4). Therefore, the reference list provided below pertains specifically to the references included in the remaining chapters of the thesis (i.e., Chapters 1 and 2).

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