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A High-Throughput Approach to Monitoring Pesticide Residues in Honey and Propolis: Applications in Albanian Beekeeping

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Abstract

Honey and other bee products are valued natural foods and important bioindicators of environmental contamination, yet systematic residue data from Albanian beekeeping producers are limited. This study aimed to characterize pesticide residues in honey and propolis to assess consumer safety and potential risks to bee health. Honey and propolis samples were collected from beekeeping producers in six Albanian regions, including one organic park, to capture managed and semi natural exposure conditions. A validated multi residue QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) based method aligned with USP <561> was applied using GC MS/MS and LC MS/MS to determine ~95 pesticides across organochlorine, organophosphate, pyrethroid and other classes, with compound specific limits of detection and regulatory decision thresholds. All honey samples complied with USP <561> limits and relevant EU maximum residue levels, indicating low dietary risk for consumers and consistency with recent international assessments of honey safety. Two propolis samples contained aldrin/dieldrin, cyfluthrin and/or fenpropathrin at concentrate above maximum residue limits. There were also legacy and current use pesticides detected at low levels in hive related products from organically managed colonies and regions, suggesting environmental contamination. These findings suggest that Albanian honey is safe for human consumption under current conditions, while highlighting potential chronic and sublethal risks to bee colonies from pesticide accumulation in hive materials. Due to simplicity, scalability, and an abundance in analyte coverage, this method may serve as a high-throughput platform for pesticide monitoring in apiculture and provides a robust framework for national residue monitoring in bee products. Bee-related products can be used as a practical bioindicator to guide pollinator protection strategies and pesticide risk management at the country and regional level.

Keywords: Pesticide residue; Honey; GC–MS/MS; LC–MS/MS; Pollinator; Pesticide

Introduction

Beekeeping remains one of Albania's most historically significant and economically vital agricultural sectors, with an estimated 290,000 and 518,790 bee colonies recorded in 2022 and 2023 respectively [1-4]. Honeybee populations found across Albania are generally characterized as hybrid subspecies derived from Apis mellifera carnica and A. m. macedonica, both of which occur in high abundance, particularly along the country's southern regions [5-7]. Given the importance of apiculture, this sector contributes

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substantially to the livelihoods of rural communities while supporting the pollination of a wide range of cultivated crops and wild flora. Bees, in particular, play a critical role, both in Albania's honey production and for enhancing overall agricultural output [3,8].

Recent assessments suggest that managed honeybee populations across the Europe, including Albania, continue to experience substantial environmental pressures, and may face maximum winter colony losses of ~22-30%, similar to those of neighboring regions [9]. Such losses raise significant concerns regarding the long-term sustainability of pollinator communities. Given the extent to which farmers depend on pollinators to support the healthy development of fruits, vegetables, and field crops [8,10], pollinator health should be a key consideration for pesticide use and distribution. Insufficient pollinator activity can markedly reduce crop yields by affecting morphological traits such as size, quality, and shape, ultimately diminishing farm profitability and competitiveness in local markets [10,11]. Bees represent a critical keystone species whose pollination services enhance yields across numerous agricultural crops [12]. They also support broader biodiversity by pollinating wildflowers and contributing to the preservation and stability of natural ecosystems [10,12].

Outside of human managed colonies, wild bees are also widespread across Albania's forests and semi natural habitats, where they play an important role in pollinating the country's Mediterranean landscapes [5,13]. The region's climate supports the natural proliferation of a diverse wild bee community [5], and Albanian farmers have historically domesticated locally occurring wild bees for agricultural use. Early evidence indicates that domesticated wild bee populations experience low survival rates when moved into or near intensively cultivated agricultural areas, largely due to elevated pesticide exposure associated with these landscapes [14,15].

Pesticide use on agricultural land is widely recognized as a major threat to both managed and wild bee populations [14-16]. Field studies have demonstrated that bees foraging within or near pesticide treated crops exhibit higher mortality rates, along with detrimental sublethal effects resulting from exposure to systemic insecticides, particularly when alternative, uncontaminated foraging resources are limited [14,15,17]. However, very little research has been conducted on pesticide residue and drift within the regions of Albania which may have downstream effects on be byproducts for human consumption.

Given the widespread use of pesticides, several analytical strategies have been developed and implemented to monitor pesticide residues in bee derived products. One widely applied approach is the QuEChERS–LC MS/MS method [18-22]. Modified QuEChERS, an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe, has been optimized for honey/honey-product analysis through the use of alternative salts, sorbents, and nano sorbents, which can be coupled with LC MS/MS or GC MS/MS to achieve multi class, multi residue detection within a single analytical run [18,20-22]. Solid phase extraction is also commonly used as a standalone clean up step prior to chromatographic analysis [23,24].

For large scale monitoring efforts, broad scope LC MS/MS and combined LC MS/MS–GC MS/MS methods are used regularly [19]. They are capable of quantifying hundreds of pesticides at low µg/kg concentrations and remain the preferred analytical approaches for high throughput, regulatory surveillance of honey [18,20-22]. Additional platforms are also applied, including ultra high-performance LC MS/MS systems, and less sensitive HPLC-UV-Vis. The latter is used for limited analyte panels, regardless this equipment demonstrates that a range of analytical options are available and can be fitted for specific monitoring objectives [25]. Furthermore, literature consistently highlights that citrate buffered and other modified QuEChERS workflows are particularly economical, adaptable, and well suited for honey and other apiculture products [19,20,21,26]. Therefore, there is continued support for their use in large-scale residue monitoring programs.

Given this framework, the present study employed a validated multi residue method based on USP <561>, incorporating QuEChERS extraction and dual GC MS/MS/LC MS/MS detection for the analysis of honey and propolis. This approach enabled the simultaneous determination of 95 pesticides that are commonly used in agriculture, including organochlorine, organophosphate, pyrethroid, and additional pesticide classes within a single analytical workflow. The method combined the use of a standardized QuEChERS extraction followed by class appropriate GC MS/MS or LC MS/MS analysis, along with the flexibility of either "cut point" single point screening or fully quantitative calibration curves. This method provides a high throughput platform suitable for routine monitoring of bee derived products and potentially other agricultural commodities [9,19,28]. Implementation of this method aligns with contemporary high throughput pesticide surveillance strategies for honey and pollen while adhering to USP decision limits, thereby supporting regulatory interpretation and ensuring comparability with international food safety standards [18,27,28].

The aim of this project was to address existing gaps in the field by generating systematic data on pesticide residues in honey and other bee derived products produced in Albania. Additionally, implications for pollinator health and food safety concerns were explored. This work supports the protection of critical pollinator populations to guide the development of sustainable pest management strategies, and ultimately contributes to strengthening agricultural productivity [10,14,19].

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Materials & Method

Sampling Methodology

Following the spring harvesting, 20 samples from honey and propolis were collected from 6 Albanian regions and geographical zones.

Analytical Method

Pesticide residues in honey and propolis were determined using a QuEChERS-based multi-residue method with GC–MS/MS and LC–MS/MS detection, adapted from USP <561> (Articles of Botanical Origin) and AOAC Official Method 2007.01 for pesticide residues by acetonitrile extraction and partitioning with magnesium sulfate (QuEChERS). Samples were prepared in a similar manner to those presented Blažková et al. Briefly, samples 2.0 ± 0.06 g of test material were weighed into 50 mL screw-capped polypropylene centrifuge tubes; samples were analyzed at Nucro-Technics Toxicology Laboratories, Toronto, Ontario, Canada. Ten millilitres of purified water were added to rehydrate samples. Process control and spiking solutions were added where applicable before tubes were vortexed and equilibrated at room temperature for approximately 15 min to increase sample surface area.

After hydration, 10 mL of 1% (v/v) acetic acid in acetonitrile were added and the tubes were shaken vigorously for 1 min by hand, vortex, or at 200 rpm on a homogenizer. QuEChERS extraction salts (6 g anhydrous $MgSO_4$ and 1.5 g anhydrous sodium acetate) were then added, the tubes were immediately shaken to break up the salts, and extraction was continued for 1 min before centrifugation at 3,000 × g for 5 min. A 1 mL aliquot of the upper acetonitrile layer was transferred to a 2 mL dispersive-SPE tube for cleanup, shaken for approximately 1 min, and centrifuged at 8,000 × g for 1 min.

For GC–MS/MS analysis, 250 µL of the cleaned supernatant were transferred to autosampler vials and fortified with 25 µL of a working internal standard solution containing d_{10}-phenanthrene and warfarin, 25 µL of 1% acetic acid in acetonitrile, and 10 µL of an analyte protectant solution (L-gulonolactone and D-sorbitol). For LC–MS/MS analysis, a separate 250 µL aliquot of the clean extract was evaporated at 45 °C for approximately 30 min and reconstituted in 250 µL of a methanol/5 mM ammonium formate/formic acid reconstitution solution; internal standards were added analogously, but analyte protectant was omitted. Matrix-matched calibration standards were prepared by spiking blank matrix extracts (200–250 µL) with calibration working solutions and internal standards to cover 0.005–2 mg/kg for each analyte, assuming a 2 g sample mass and the described extraction scheme.

GC–MS/MS measurements were carried out on an Agilent 7890B/7010 system equipped with a mid-column backflush configuration and two HP-5ms UI columns (15 m × 0.25 mm, 0.25 µm film), operated in electron impact MRM mode with a total run time of 23.75 min. LC–MS/MS measurements were performed on a Thermo Q Exactive with a Dionex Ultimate 3000 HPLC using a Kinetex F5 column (5 µm, 4.6 × 250 mm) at 45 °C, a 25 min gradient between mobile phases A and B (0.5% formic acid in 5 mM ammonium formate/methanol mixtures), and full-scan SIM/dd-MS^2 acquisition for the target pesticides. Quantification was based on matrix-matched calibration using a linear regression model with $1/x^2$ weighting and internal standard correction; where required, the procedure could be operated in screening (single-point "cut-point") mode at the relevant maximum residue limits.

Ninety-five pesticides were quantified from one sample and included in Table 1.

Table 1: List of ~95 pesticides analyzed with analytical platform, USP <561> maximum residue limits, and limits of detection achieved in the test batch.

Target Compounds	Analytical Instrument	USP <561> Limits(µg/g)	LoD in Test Batch (µg/g)
Acephate	LCMS-MS	0.1	0.005
Alachlor	GCMS-MS	0.05	0.05
Aldrin and dieldrin	GCMS-MS	0.05	0.05 and 0.05
Azinphos-ethyl	GCMS-MS	0.1	0.01
Azinphos-methyl	GCMS-MS	1	0.01
Bromophos-ethyl	GCMS-MS	0.05	0.05
Bromophos-methyl	GCMS-MS	0.05	0.005
Bromopropylate	GCMS-MS	3	0.005
Chlordane (sum of cis-, trans-, and oxychlordane)	GCMS-MS	0.05	0.005
Chlorfenvinphos	GCMS-MS	0.5	0.05
Chlorpyriphos-ethyl	GCMS-MS	0.2	0.005
Chlorpyriphos-methyl	GCMS-MS	0.1	0.005

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Chlorthal-dimethyl	GCMS-MS	0.01	0.005
Cyfluthrin (sum of)	GCMS-MS	0.1	0.005
λ-Cyhalothrin	GCMS-MS	1	0.005
Cypermethrin and isomers (sum of)	GCMS-MS	1	0.005
DDT (sum of o,p′-DDE, p,p′-DDE, o,p′-DDT, p,p′-DDT, o,p′-TDE, and p,p′-TDE)	GCMS-MS	1	0.005
Deltamethrin	GCMS-MS	0.5	0.05
Diazinon	GCMS-MS	0.5	0.05
Dichlofluanid	GCMS-MS	0.1	0.005
Dichlorvos	GCMS-MS	1	0.01
Dicofol	GCMS-MS	0.5	0.10 and 0.05
Dimethoate and omethoate (sum of)	LCMS-MS	0.1	0.005 and 0.005
Dithiocarbamates (Expressed as CS_2)	GCMS-MS	2	0.04
Endosulfan (sum of isomers and endosulfan sulphate)	GCMS-MS	3	0.05 and 0.005
Endrin	GCMS-MS	0.05	0.01
Ethion	GCMS-MS	2	0.005
Etrimphos	GCMS-MS	0.05	0.005
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	GCMS-MS	0.1	0.005 and 0.005
Fenitrothion	GCMS-MS	0.5	0.005
Fenpropathrin	GCMS-MS	0.03	0.01
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxon sulfone, and fensulfothion sulfone)	GCMS-MS	0.05	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon sulfone, fenthion-oxon sulfoxide,fenthion sulfone, and fenthion-sulfoxide)	GCMS-MS	0.05	0.005
Fenvalerate	GCMS-MS	1.5	0.05
Flucythrinate	GCMS-MS	0.05	0.005
τ-Fluvalinate	GCMS-MS	0.05	0.01
Fonophos	GCMS-MS	0.05	0.005
Heptachlor (sum of heptachlor, cis-heptachlorepoxide, and trans-heptachlorepoxide)	GCMS-MS	0.05	0.010, 0.010 and 0.010
Hexachlorobenzene	GCMS-MS	0.1	0.005
Hexachlorocyclohexane (sum of isomers α-, β-, δ-, and ε-)	GCMS-MS	0.3	0.005,0.005 and 0.005
Lindane (γ-hexachlorocyclohexane)	GCMS-MS	0.6	0.005
Malathion and malaoxon (sum of)	GCMS-MS	1	0.005 and 0.005
Mecarbam	GCMS-MS	0.05	0.05
Methacriphos	GCMS-MS	0.05	0.005
Methamidophos	LCMS-MS	0.05	0.005
Methidathion	GCMS-MS	0.2	0.005
Methoxychlor	GCMS-MS	0.05	0.005
Mirex	GCMS-MS	0.01	0.005
Monocrotophos	LCMS-MS	0.1	0.1
Parathion-ethyl and paraoxon-ethyl	GCMS-MS	0.5	0.005 and 0.200
Parathion-methyl and paraoxon-methyl	GCMS-MS	0.2	0.005 and 0.05
Pendimethalin	GCMS-MS	0.1	0.005
Pentachloranisole	GCMS-MS	0.01	0.005

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Permethrin and isomers (sum of)	GCMS-MS	1	0.05
Phosalone	GCMS-MS	0.1	0.005
Phosmet	GCMS-MS	0.05	0.005
Piperonyl butoxide	GCMS-MS	3	0.005
Pirimiphos-ethyl	GCMS-MS	0.05	0.005
Pirimiphos-methyl (sum of pirimiphos-methyl and N-desethyl-pirimiphos-methyl)	GCMS-MS	4	0.005
Procymidone	GCMS-MS	0.1	0.005
Profenophos	GCMS-MS	0.1	0.05
Prothiophos	GCMS-MS	0.05	0.005
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I, and pyrethrin II)	LCMS-MS	3	0.01
Quinalphos	GCMS-MS	0.05	0.005
Quintozene (sum of quintozene, pentachloraniline, and methyl pentachlorphenyl sulfide)	GCMS-MS	1	0.005 and 0.05
S-421	GCMS-MS	0.02	0.005
Tecnazene	GCMS-MS	0.05	0.005
Tetradifon	GCMS-MS	0.3	0.005
Vinclozolin	GCMS-MS	0.4	0.005

Table 2: Specific samples with pesticide residues exceeding USP <561> maximum residue limits from intensive agriculture zones.

Propolis Sample	Pesticide Class	Pesticide	Result (µg/g)	Acceptance criterion / limit (µg/g)	Status
KU-2	Pyrethroid	Fenpropathrin	0.3844	0.03	FAIL
KU-E4	Organochlorine	Aldrin and dieldrin	1.2255 (total)	0.05 and 0.05	FAIL
KU-E4	Organochlorine	Cyfluthrin (sum of isomers)	0.2415	0.1	FAIL
KU-E4	Pyrethroid	Fenpropathrin	0.4726	0.03	FAIL
KU-E4	Organophosphates	Parathion-methyl and paraoxon methyl	0.3073 (total)	0.2	FAIL
KU-MS	Pyrethroid	Fenpropathrin	0.4627	0.03	FAIL

Table 3: Pesticide residues detected at acceptable levels in honey from an organically managed colony

Pesticide	Class	Example conc. (µg/g)	USP <561> MRL (µg/g)	Agricultural Use	Bee Toxicity
Bromopropylate	Organochlorine	trace (<0.005)	3	Acaricide (banned)	Moderate–High
Chlordane (sum)	Organochlorine	~0.001	0.05	Insecticide (banned)	Highly toxic
Chlorpyrifos-methyl	Organophosphate	~0.001	0.1	Insecticide	Highly toxic
Cyfluthrin (sum)	Pyrethroid	~0.002–0.003	0.1	Insecticide (restricted)	Highly toxic
Cypermethrin (sum)	Pyrethroid	~0.001–0.002	1	Insecticide	Highly toxic
DDT (sum)	Organochlorine	~0.001	1	Insecticide (banned)	Highly toxic
Endrin	Organochlorine	~0.01	0.05	Insecticide (banned)	Highly toxic
Lindane (γ-HCH)	Organochlorine	~0.001	0.6	Insecticide (banned)	Highly toxic
Malathion (sum)	Organophosphate	~0.006	1	Insecticide	Moderate–High
Methidathion	Organophosphate	~0.002	0.2	Insecticide/acaricide	Highly toxic
Methoxychlor	Organochlorine	~0.002	0.05	Insecticide (banned)	Moderate–High
Parathion-methyl (sum)	Organophosphate	~0.001	0.2	Insecticide (banned)	Highly toxic
Pentachloroanisole	Organochlorine	trace	0.01	Metabolite of fungicide quintozene	Low–Moderate

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Pirimiphos-ethyl	Organophosphate	~0.001	0.05	Insecticide	Moderate–High
Prothiophos	Organophosphate	~0.002	0.05	Insecticide	Highly toxic
Quinalphos	Organophosphate	~0.001–0.002	0.05	Insecticide	Highly toxic
Quintozene (sum)	Organochlorine	~0.001	1	Fungicide	Low–Moderate

Results

Pesticide Detection Overview

Of the 95 pesticides analyzed across 20 honey and propolis samples from six Albanian regions, the majority of samples tested showed pesticide residues below USP <561> maximum residue limits (MRLs). However, three propolis samples exhibited levels above MRL of pyrethroid and organochlorine insecticides, two of which were from the intensively farmed region located near industrial areas (Table 2). Similarly, one organically managed sample had detectable concentrations of multiple legacy and contemporary pesticides below the MRL (Table 3), consistent with residue patterns reported for honey and hive products in other regions [29-31].

Three propolis samples failed to meet regulatory standards. Sample KU-E4 contained three pesticides exceeding their respective USP<561> MRLs: aldrin/dieldrin (organochlorine) was ~24.5x higher, cyfluthrin (pyrethroid) was ~2.4x higher, and fenpropathrin (pyrethroid) was ~15.8x higher (Table 2). Sample KU-2 and KU-MS from the same region exceeded limits for fenpropathrin by ~12.8x and 15.4x respectively. This suggests an area of contamination potentially related to intensive local pesticide use (Table 2). Similar patterns of pyrethroid and organochlorine contamination have been reported in honey and other hive products from intensive agricultural areas [29,30].

Discussion

The presence of multiple banned organochlorine pesticides (chlordane, DDT, endrin, lindane, methoxychlor) in honey from an organically certified apiary underscores the environmental persistence and long-range transport of these compounds [32-37]. This finding is notable given that synthetic pesticide application is prohibited in certified organic apiaries and no supplementary sugar feeding is provided during the spring season. Organochlorines applied decades ago remain detectable in soils, sediments, and vegetation due to their lipophilicity, resistance to microbial degradation, and strong tendency to bioaccumulate in food webs [38,39]. Consequently, these compounds are routinely reported at low concentrations in honey from diverse geographic regions, including areas with relatively low current pesticide use [32-37].

Fenpropathrin is a synthetic pyrethroid insecticide that is not approved for use in the European Union under Regulation (EC) No 1107/2009 [40]. It remains under regulatory scrutiny because of its high acute toxicity to bees and its potential to cause sublethal neurotoxic effects [41]. Laboratory and semi field studies demonstrate that fenpropathrin significantly reduces honeybee survival, learning and memory, and homing ability at concentrations overlapping with field exposures, indicating serious risks to colony health even in the absence of immediate mortality [41-43].

Cyfluthrin, another synthetic pyrethroid, is recognized for its high toxicity to aquatic organisms and strong neurotoxic effects in insects [44,45]. As a result, its use has been restricted or phased out by many regulatory authorities [46]. Both aldrin and dieldrin are persistent organochlorine insecticides that have been banned internationally under the Stockholm Convention [47] due to their long environmental half-lives, bioaccumulation in fatty tissues, and associations with carcinogenic and neurotoxic effects in humans and wildlife [32,47]. The detection of these legacy compounds in propolis samples reflects their continued environmental persistence decades after prohibition, a pattern widely documented for organochlorines in soils, sediments, and flora [32-35].

The co-occurrence of multiple pesticide residues in propolis collected from intensive agricultural zones suggests cumulative exposure pathways and highlights propolis as a particularly sensitive matrix for environmental monitoring [48]. Propolis is a resinous material collected by bees from plant exudates and buds [49] and has a strong tendency to concentrate lipophilic contaminants. Recent studies indicate that beehive products can accumulate pesticide residues to levels that may pose dietary risks for consumers, particularly when such products are consumed as concentrated nutraceutical preparations [48,50-52].

A honey sample from an organically managed apiary revealed 21 pesticide residues at detectable concentrations (Table 3), all below their respective maximum residue limits (MRLs). The detected compounds were predominantly legacy organochlorines (e.g., chlordane, DDT metabolites, endrin, lindane, methoxychlor) and organophosphates (e.g., chlorpyrifos methyl, fenitrothion, malathion, methidathion, parathion methyl), with trace amounts of pyrethroids (cyfluthrin, cypermethrin). This multi class residue profile mirrors those reported in honey from other Mediterranean and temperate regions [29,30,36,37].

The presence of contemporary organophosphates and pyrethroids at trace levels in organic honey is plausibly explained by atmospheric drift, which can transport pesticides from neighboring conventional farms, from pollinators

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foraging on wildflowers or crops adjacent to treated fields, or through residual contamination of equipment or storage containers [48, 53]. Honeybees typically forage within a 3-5 km radius of the hive; therefore, even organically managed colonies located near intensive agriculture may experience pesticide exposure through contaminated nectar, pollen, and water sources [54-56].

In contrast to organic honey, no pesticide residues were detected in conventionally managed honey from the same study, where colonies were supplemented with sugar syrup rather than relying exclusively on floral resources. Similar patterns of reduced environmental residue burdens have been observed in colonies with limited foraging activity or extensive feeding, where nutritional management, foraging distance, and landscape context jointly influence pesticide exposure [57,58]. Differences in stored honey volume and comb turnover may further dilute or concentrate residues, potentially contributing to the observed variation [59,60].

In the present analysis, all honey samples complied with existing regulatory limits. However, the detection of legacy organochlorines, highly toxic organophosphates, and pyrethroids, even at trace concentrations, raises concerns for both pollinator health and food safety [32-35, 52,51]. The detection of legacy pesticides is a common finding in propolis samples (Blažková et al.). While the specific pesticides identified in this study were not necessarily reported in other investigations, this variability likely reflects regional differences in historical and current pesticide use. Regardless of the compounds detected, pesticide contamination of bee bread and other bee products represents a potential risk for both consumers and apiaries.

Chronic dietary exposure to mixtures of pesticides may pose non carcinogenic and carcinogenic risks that are often underestimated when residues are assessed individually, as demonstrated in recent risk assessments of beehive products [52,51]. For honeybees, sublethal pesticide exposure can impair navigation, learning, immune function, and brood development, contributing to colony decline even when residue levels fall below acute LD_{50} based thresholds [14,17,48,61,62]. Synergistic interactions among pyrethroids, organophosphates, neonicotinoids, and certain fungicides can further amplify toxicity beyond simple additivity, a phenomenon documented in both laboratory and field studies [63-65]. The elevated propolis contamination observed in intensive agricultural zones in this study is consistent with regional and global reports of reduced wild bee survival and increased colony losses when hives originating from semi natural or forested habitats are relocated near pesticide treated crops [29,30]. Pesticide exposure may also pose risks to consumers and commercial brands that are unaware of pesticide residues present in organic bee products such as honey and propolis.

The validated QuEChERS–GC–MS/MS–LC–MS/MS method used in this study enabled the quantification of 95 pesticides with limits of detection (LoDs) ranging from 0.005 to 0.2 µg/g, providing sensitivity suitable for regulatory surveillance and risk assessment in honey and propolis [66,67]. Matrix matched calibration and internal standard correction ensured accurate quantification across diverse pesticide classes. The dual platform approach, GC–MS/MS for semi volatile and non-polar analytes and LC–MS/MS for polar and thermally labile compounds, achieved analytical coverage comparable to large scope monitoring methods reported for honey and pollen in Europe and other regions [48,66-68]. This configuration aligns with current best practices for multi residue pesticide analysis in apicultural matrices and supports its application in ongoing surveillance at national and regional levels [48,67,68].

The use of dual platform GC MS/MS and LC MS/MS analysis has previously been demonstrated for pesticide determination in bee bread [69-71]. The modified analytical approach applied in this study demonstrates that similar methodologies can be effectively extended to other bee by products, such as honey and propolis, thereby enabling a more comprehensive assessment of pesticide contamination in consumer products following dilution and processing by bee colonies.

Future Directions/Implications

Increased testing of bee products by the government or relevant testing bodies should be done to better understand the possible contamination and residue left by pesticide use in the area. Furthermore, apiaries should be aware that their products may be contaminated unknowingly by nearby agricultural practices beyond their control. Additional testing can be used to detect high-risk regions of the country and help prevent pesticide contamination via pollution or atmospheric drift. Test results should also be published to allow consumers to know the risks associated with their purchases, and to help the public understand that organic products may have pesticide contamination.

Conclusion

In conclusion, pesticide residues in Albanian honey were largely within regulatory limits, suggesting low acute dietary risk. Yet, the detection of banned organochlorines and highly toxic pyrethroids, especially in propolis from intensively farmed areas suggests ongoing environmental exposure to toxicologically relevant compounds. The co-occurrence of multiple pesticide classes, including substances with known neurotoxic and sublethal effects on pollinators, highlights the importance of considering mixture toxicity and chronic exposure in risk assessments. These findings support the use of propolis as a sensitive bioindicator of environmental contamination and underscores the need for

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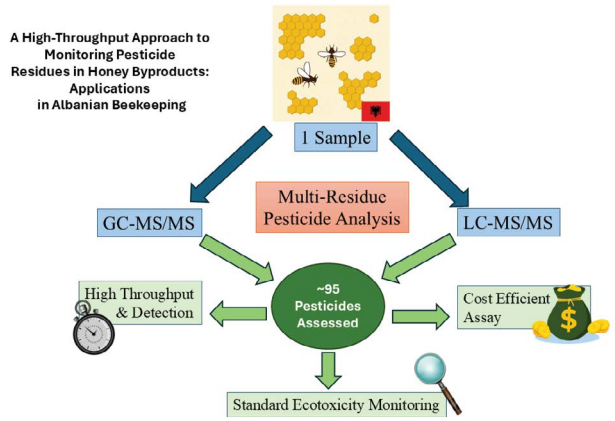

Figure 1: Schematic overview of the high-throughput multi-residue analytical workflow used to assess approximately 95 pesticide residues in Albanian honey byproducts using combined GC-MS/MS and LC-MS/MS platforms from a single sample, enabling rapid, cost-efficient, and standardized ecotoxicological monitoring."

continued toxicological surveillance using high-throughput methods. The method used in this paper is the first instance of identifying ~95 pesticides from a single sample of honey or propolis, to our knowledge and can better inform pollinator protection and human health risk evaluation.

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