Review Article

A Comprehensive Narrative Review on the Hazards of Bee Honey Adulteration and Contamination

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Honey bees are renowned for producing a remarkable substance known as bee honey, which stands as a functional food celebrated for its numerous health benefits. This natural wonder possesses a spectrum of advantageous properties, including anti-inflammatory, antioxidant, analgesic, antibacterial, and wound-healing qualities. However, in our modern era of heightened utilization of bee products, a new and pressing global health concern has emerged—the contamination of honey with pesticides, antibiotics, microorganisms, and heavy metals. The consumption of beekeeping products containing pesticide residues has been linked to a range of health issues, including genetic malformations, cellular degradation, allergic reactions, and even potential carcinogenic effects. Troublingly, documented cases exist of botulism in newborns resulting from the ingestion of contaminated honey. Additionally, the use of antibiotics in beekeeping practices has been associated with the concerning emergence of antibiotic resistance. This comprehensive review sheds light on the substantial consequences of honey contamination for human health. It underscores the urgent need for the establishment of a rigorous monitoring system, the validation of minimum acceptable pollutant levels, and, at the very least, the regulation of maximum residue limits for bee products, with a particular emphasis on bee honey.

1. Introduction

Functional foods offer the potential for substantial health benefits, serving not only as a source of essential nutrients but also as a preventive measure or therapeutic intervention for specific medical conditions. Functional foods may have positive health benefits because they contain bioactive components with specific biological substance properties [1].
Even though the functional food business is one of the fastest-growing industries in Europe, those foods are not specifically regulated in the region [2].

Honey is naturally produced by bees (Apis mellifera L.), and it is described as a sweet substance derived from plant nectar or secretions of living plant components that have been dried. It has long been recognized as food with functional qualities that have been demonstrated through studies and clinical trials to promote a healthy lifestyle [1, 3].

Since there is a substantial honey trade among the European Union (EU) nations, a special regulation that addresses the unique properties of honey is in place to govern the quality of honey. Therefore, honey from EU countries or sold on its territories must meet certain specific compositional requirements related to its carbohydrate content, humidity, acidity, electrical conductivity, diastase activity, and hydroxymethylfurfural content (HMF) to protect the authenticity, safety, and quality of honey [4].

Unfortunately, throughout production, honey can be falsified, for instance, by adding glucose solutions, sucrose syrups, corn syrup, high fructose corn syrup, inverted sugar syrup, or sugar cane juice, or by deploying improper beekeeping techniques [4–6]. Anthropogenic contaminants from the environment (e.g., metal traces, polychlorobiphenyls, and pesticides from doused or rotating crops), as well as improper beekeeper’s management, can contaminate honey (e.g., medicines and insecticides used to treat the colony) [4].

As a result, the consumption of honey raises concerns about the health of the population and the safety and quality of the product in terms of the number of contaminants it contains, especially harmful trace elements [4].

Consumers can obtain valuable information from the conclusions of the analysis of the extraordinary therapeutic properties of bee honey and the risk to which they are exposed following the consumption of contaminated or adulterated honey [4].

Given the rise in interest in honey’s elemental analysis over the past few years, it is unsurprising that honey and its quality are the subjects of several research publications. This review highlights the importance of analyzing honey’s quality and identifies possible contamination causes.

2. Strategies and Methods Used to Collect
   Data from the Literature

The data presented in this review were obtained through literature analysis (MEDLINE, PubMed, Google Scholar, OMIM, and MedGen databases) using the following keywords: honey adulteration, honey contamination, pesticides, insecticides, antibiotics, xenobiotics, microorganism, pathogens, heavy metals, or Apis mellifera.

3. Honey: A Functional Food

3.1. Composition of Honey. Honey is a nutrient-rich, liquid food. Its composition includes fructose and glucose (≥80%), water (≥16%), ash (0.2%), and amino acids (<0.1%), with trace amounts of enzymes, antioxidants, vitamins (e.g., B1, B2, B3, B6, B9, C, and K), phenolic compounds, minerals (e.g., Na, Ca, K, Mg, P, Se, Cu, Fe, Mn, Cr, and Zn), and other chemicals compounds [7]. The botanical and geographic sources of the product significantly influence its chemical composition and health benefits [8].

Honey contains flavonoids from nectar, pollen, and plant resins collected by honey bees. Flavonoids and phenolic acid are the main polyphenolic components of honey, and they are responsible for preventing oxidation due to their capacity to decrease free radical production and scavenge radicals. Also, flavonoids significantly improve honey products’ antibacterial and antifungal value, being responsible for the anti-inflammatory, antioxidant, and antimutagenic effects. Bee salivary enzymes (e.g., glucose oxidase) and peptides (e.g., defensin−1) also contribute to the antibacterial properties of honey, and they ensure its microbiological stability [1, 8, 9]. Despite the complexity of honey’s chemical composition, hydrogen peroxide remains a crucial compound responsible for its antimicrobial action [10, 11].

3.2. Health Benefits. Honey was the first sweetener used by humans. Ancient civilizations universally acknowledged several advantages of frequent honey intake, such as treating cardiovascular and gastrointestinal illnesses and pleasant organoleptic characteristics of honey [12–15]. As a result, honey has been established as one of the most essential treatments in natural medicine [1].

Bee honey’s medical value has been studied in both humans and animals. Based on the in vivo results on rats, those who consumed altered honey underwent significant changes, including weight growth and increased levels of circulating glucose, triglycerides, and cholesterol. Over time, that could lead to liver and renal disease [16, 17].

Bee honey has been used for centuries to treat colds, sore throats, and coughs due to its anti-inflammatory and immune-boosting characteristics. Specialized literature places a strong emphasis on honey’s anti-inflammatory [18], antioxidant [19], and anticarcinogenic capabilities against breast and colon cancer [20]. Either oral ingestion or topical use of honey can provide a therapeutic effect on human health. When consumed orally, linden honey relieves fevers and stomach pains and prevents migraines, whereas lavender honey treats coughs and sore throats; acacia honey is an excellent sedative and tonic; mint honey is a great anesthetic, anticinematic, and tonic; wildflower honey has a strong antibacterial effect; fir honey and sunflower honey are helpful in respiratory disorders for fluidizing bronchial secretions; mountain honey provides potential advantages in allergies and pulmonary diseases [21]. Honey is excellent for healing nocturnal cough brought on by an upper respiratory tract infection by reducing its frequency and intensity and improving both children’s and parents’ sleep quality [9]. But even so, due to the newborns’ weakened immunity against Clostridium botulinum, a potential honey contaminant, the consumption of honey in infants under 12 months is banned. Complex natural substances represent an innovative approach to cough management. They create a film that
covers the oropharynx, thus acting as a mucoadhesive physical barrier instead of suppressing cough by engaging with specific receptors [22]. When consumed orally, Manuka honey has been shown to have a range of health benefits, including soothing sore throats, aiding in digestion, and boosting the immune system. Additionally, Manuka honey has been found to have anti-inflammatory properties, making it a potential treatment option for conditions such as inflammatory bowel disease and arthritis. When applied topically, Manuka honey has been shown to have wound-healing properties, making it a popular choice for treating burns, cuts, and other skin conditions [23, 24].

Bee honey is formulated in syrups in combination with extracts of certain plants such as Grindelia robusta, Plantago lanceolata, and Helichrysum italicum, which have protective, demulcent, anti-inflammatory, and adjuvant cytoprotective properties [9].

At the same time, the benefits of topical therapy with honey have been noticed in the treatment of athlete’s foot, vaginal lesions, burns, lip injuries, and eczema [3].

Due to its anti-inflammatory and antioxidant properties, honey benefits metabolic syndrome. Six months of daily honey consumption of 15 g was associated with weight loss, improved lipid metabolism, and reduced levels of triglycerides and cholesterol [1, 23].

Furthermore, it has been suggested that honey can relieve digestive issues such as gastroenteritis-related diarrhea. Due to its bactericidal properties (reduced colonization of Enterobacteriaceae and improved colonization with probiotic bacteria (Bifidobacterium and Lactobacillus)), orally ingested honey appears to be a proper adjuvant therapy in acute diarrhea in children and adults, reducing the duration of diarrhea episodes [1].

3.3. Potential Health Risks of Consuming Honey. Currently, there are multiple honey varieties, either monofloral or polyfloral. Among the types of monofloral honey, we mention acacia honey from Robinia pseudoacacia L., chestnut honey from Castanea sativa Mill., clover honey from Trifolium pratense L., dandelion honey from Taraxacum officinale, eucalyptus from Eucalyptus spp., lavender honey from Lavandula angustifolia Mill., linden tree honey from Tilia cordata Mill., orange honey from Citrus spp., pine honey from Pinus spp., raspberry honey from Rubus idaeus L., rhododendron honey from Rhododendron spp., rosemary honey from Rosmarinus officinalis L., strawberry tree honey from Arbutus unedo L., sunflower honey from Helianthus annuus L., thyme honey from Thymus spp., etc. [25].

Regarding the bee species used by beekeepers, only two of the Apis species (A. mellifera and Apis cerana) are used for commercial reasons. That is a consequence of the characteristics of some species, such as giant and dwarf bees, which prefer to nest outside and cannot be housed in artificial hives. The species A. cerana is more productive in bee honey production than other species [18].

Flowers are the bee’s main sources of nutrition. Traces of unsuitable metals and insecticides can be identified on their surface, which the bees subsequently introduce into the hive through nectar and pollen they collect and transport on their bodies. Contaminants are concentrated during the maturation process of honey (enzymatic conversion of sucrose and reduction of water). Due to pest control treatments of hives or through the transfer of contaminated beeswax, veterinary medicinal drugs (such as antibiotics banned in beekeeping or legal acaricides) can be found in honey [26–30]. At the same time, honey must not contain traces of metals or the heavy metal content should be minimal, as defined by EU food regulations [8, 31–33].

Among the samples of bee honey monitored annually by the EU, cadmium (Cd) is the residue that was detected in the highest concentration, followed by lead (Pb), copper (Cu), numerous antibiotics, organophosphates organochlorines, and other pesticides [8].

By implementing ecological agriculture standards, employing appropriate beekeeping techniques, keeping the hives far enough from potential sources of contamination, and limiting treatment against mite pests, honey residues can be kept to a minimum [8].

4. Honey Adulteration

One of Europe’s top ten most adulterated food products is honey, which is also placed third on the list of food fraud victims in the United States Pharmacopoeia food fraud database, after milk and olive oil. Adulteration is incorporating foreign substances into food, often done to increase quantity at the expense of quality [34–36]. Honey falsification is the strategy of decreasing manufacturing costs while increasing profits. Adulterated honey leads to economic effects by decreasing its price [37].

Bee honey can be altered by using store-bought syrups and inexpensive sweeteners. Cane sugar, beet sugar, glucose syrup, fructose syrup, corn syrup, inverted syrup, and high fructose inulin syrup are the most widely recognized adulterants. Figure 1 provides an overview of the chemical structures of some frequently reported sugar adulterants.

Adding those sweeteners affects bee honey’s chemical and biochemical activity, including its enzymatic activity, electrical conductivity, and special component concentration [38, 39]. It has been noticed that the choice of adulterants relies on the geographic area, the financial benefits, and the accessibility of acquiring them. For instance, producers from Turkey and France use wheat and rice syrup as adulterants, unlike other EU producers who adulterate honey with high fructose inulin syrup [39, 40].

There are three types of honey adulteration: direct, indirect, and blending. Direct adulteration is a postproduction process that involves adding sweeteners to honey in varying amounts (7%, 15%, and 30%) to raise sweetness. Indirect adulteration entails overfeeding bees with pesticides and synthetic sweeteners to extract additional honey from the hives. Another method of altering honey is blending, which is achieved by diluting pure, high-quality honey with cheaper, low-quality honey [3].

The adulteration process reduces the antibacterial effects of pure honey and increases blood glucose levels, followed by insulin release, abdominal weight, and blood cholesterol...
levels [41, 42]. In addition to producing \( \text{H}_2\text{O}_2 \) and fructose, insulin also causes a rise in uric acid by activating the plasma membrane enzyme system with NADPH-oxidase characteristics. Atherosclerosis, diabetes, obesity, high blood pressure, coronary heart disease, and even heart failure are chronic diseases that are brought on by the simultaneous production of reactive oxygen species by glucose and fructose from sugar [39, 43].

Notwithstanding, it might be challenging to identify those kinds of adulterants because some studies have found sugar or syrup residue quantities that are equal to those found in pure honey [44]. The authenticity of the honey samples was assessed using multiple analyses: principal component analysis (PCA) and linear discriminant analysis (LDA) for physicochemical and rheological examination, high-performance liquid chromatography (HPLC), gas chromatography with mass spectrometry (GC-MS), micellar electrokinetic capillary chromatography (MEKC), and voltammetry [45–47]. Isotope ratio mass spectrometry (IRMS) is the most commonly employed approach for honey analysis, although nuclear magnetic resonance (NMR) is more reliable because it involves less sample preparation and delivers results faster. It is useful to carry out a 1H NMR investigation with statistical analysis to distinguish honey from various botanical and geographic origins. The NMR approach includes magnetic resonance to identify the molecule’s structure [37].

5. Honey Contamination

Honey contamination refers to foreign substances or contaminants that are not naturally part of the honey or are present in quantities exceeding acceptable limits. Honey can become contaminated at various stages, including during production, processing, transportation, and storage. Contaminants can come from environmental sources, beekeeping practices, or external factors. Contamination of honey can negatively affect human health, mainly if it involves harmful substances. Adulteration or contamination of honey with other substances may trigger allergic reactions in sensitive individuals. Also, long-term consumption of contaminated honey can pose health risks and cause acute or chronic health issues [48–51].
5.1. Pesticides. One of the most significant threats originating from honey contamination is the presence of pesticide residues. Bees, as pollinators, may inadvertently collect nectar from plants exposed to pesticides, transferring those harmful chemicals into the honey they produce. Long-term consumption of pesticide-contaminated honey may lead to a range of health issues, including neurological disorders, hormonal imbalances, and an increased risk of certain cancers. As those pesticides accumulate in the human body over time, their effects can be insidious, emphasizing the urgency of monitoring and controlling pesticide use in beekeeping practices [27, 31, 33, 53, 54].

To avoid bee diseases and pests, pesticides are employed worldwide; for the most part, their application is unregulated and performed without following recognized procedures. Their use is supposed to protect crops and boost agricultural output [53]. Nevertheless, uncontrollable applications can contaminate humans, animals, and the environment. Acaricides, organic acids, insecticides, fungicides, herbicides, and bactericides are a few pesticide residues that may have a carcinogenic effect on individuals. The contamination of honey and other hive products is a danger associated with using pesticides within hives [53].

Pesticides can be potentially hazardous to individuals depending on the chemical’s toxicity, the length of exposure, and the severity of the effects. Chemical compounds can bioaccumulate, and their effects may amplify in the body, generating bioconcentrations. Due to their underdevelopment and small size, children are most susceptible to pesticide contamination. Pesticide exposure can cause issues ranging from a minor skin rash to congenital malformations, neoplasms, genetic mutations, hematological disorders, and sometimes even coma or death. The endocrine, reproductive, and immune systems can be harmed by many persistent organic pollutants (POPs), including aldrin, dieldrin, heptachlor, chlordane, and hexachlorobenzene. POPs are prohibited due to the severe complications they cause after chronic exposure, though some are still in use [53, 63].

Varroacides accumulate in beewax and pollen and are the main sources of pesticide residues. Maximum residue limits (MRLs) have been established at levels expressed as parts per billion for several pollutants [52]. Various national authorities have set MRLs in honey, but the absence of consistent regulation affects international marketing and commerce [53]. For instance, the MRLs for amitraz, bromopropylate, coumaphos, cyamizole, flumethrin, and fluvalinate established by Switzerland, Germany, and Italy differ from those set by the EU, which regulated MRLs for amitraz ($0.2 \text{mg} \cdot \text{kg}^{-1}$), coumaphos ($0.1 \text{mg} \cdot \text{kg}^{-1}$), and cyamizole ($1 \text{mg} \cdot \text{kg}^{-1}$). On the other hand, the US Environmental Protection Agency has implemented MRLs for amitraz ($1 \text{mg} \cdot \text{kg}^{-1}$), coumaphos ($0.1 \text{mg} \cdot \text{kg}^{-1}$), and fluvalinate ($0.05 \text{mg} \cdot \text{kg}^{-1}$) [53].

The most popular extraction and purification approach used to detect honey pesticides is liquid-liquid extraction (LLE). Nevertheless, LLE requires a lot of sample handling steps, big sample volumes, and hazardous organic solvents. Another innovation in that direction was using low-temperature liquid-liquid extraction (LLE-LTP) in detecting deltamethrin and cypermethrin in dairy. Studies employ that technique to extract chlorpyrifos, cypermethrin, deltamethrin, and k-cyhalothrin from honey [54].

Solid phase extraction (SPE) is used to retain certain analytes on absorbents and subsequent elution of those analytes with the appropriate solvents. That technique combines the extraction and cleanup operations into a single process, resulting in clean extracts that can be analyzed rapidly through GC or LC. That method is attractive for detecting pesticides in bee honey because of its simplicity, precision, and minimal solvent usage [54].

The principle behind magnetic solid phase extraction (MSPE) is using magnetic or magnetizable adsorbents. MSPE extracts the analyte by introducing a magnetic adsorbent to a suspension or solution. The adsorbed analyte is then recovered using a suitable magnetic separator. Magnetic nanoparticles are frequently utilized as adsorbents in MSPE because, compared to conventional SPE adsorbents, they have a bigger surface area and distinct magnetic characteristics [54].

Gas chromatography is an effective method for assessing contaminant levels in complex matrices. It is frequently used to analyze pesticides in honey, in conjunction with several detection methods, including MS, MS/MS, nitrogen phosphorus detector (NPD), electron capture detector (ECD), atomic emission detector (AED), and flame photometric detector (FPD). Mass spectrometry is the most efficient pesticide detection method because it offers structural details that provide exhaustive confirmation, which is necessary for a multi-residue investigation [54].

For pesticide identification in honey, liquid chromatography (LC) is frequently employed, especially for thermally labile chemicals. Pesticides can be detected in complex matrices in low quantities using LC-MS. That system adds structural information, enhances sensitivity, and lessens matrix interference. In recent years, LC-MS/MS methods have been successfully used to determine if residues are present in honey [54].

To identify several classes of pesticides (pesticides, biocides, and other veterinary drugs) in honey, ultra-high-performance liquid chromatography (UHPLC-MS/MS) can be performed [54].
5.2. Antibiotics. In some cases, beekeepers use antibiotics to treat diseases in bee colonies, inadvertently contaminating the honey [53, 56]. The consumption of honey contaminated with antibiotic residues poses a concerning challenge to public health: the emergence of antibiotic-resistant bacteria [30, 56]. Regular intake of antibiotics through contaminated honey can contribute to the development of antibiotic-resistant strains, rendering those vital medications less effective in treating bacterial infections in humans [30, 53, 55–57]. The widespread use of antibiotics leads to an accumulation of antibiotic residues in honey, thus leading to decreased quality and challenging marketing [53, 65, 66]. Skin rashes, dermatitis, gastrointestinal symptoms, and anaphylaxis are side effects of lactam antibiotics, even in the relatively low levels that may be found in honey [64, 65]. Also, individuals are more susceptible to developing cancer after exposure to nitrofurans and nitroimidazoles [67, 68].

The Paenibacillus (Bacillus) larvae and Melissococcus plutonius bacteria that cause European and American foulbrood in honeybees are typically treated with oxytetracycline [66]. Tetracycline resistance has been reported in those bacteria, widely believed to result from its widespread usage [69]. Streptomycin, erythromycin, lincomycin, sulfonamide, and chloramphenicol are other antibiotics beekeepers use [57, 70–72].

An aminoglycoside called streptomycin is utilized in several countries, especially in Central and South America, for treating both American foulbrood (caused by P. larvae) and European foulbrood (caused by Melissococcus plutonius) [66]. Streptomycin residues were detected in those areas and in various dietary items such as milk, viscera, and meat [69, 73]. Streptomycin is currently prohibited in many countries due to its increased toxicity, even though it is frequently recommended in bee forums and beekeeping guides [57, 73].

Erythromycin is a prevalent chemotherapeutic antibacterial drug used in human and veterinary medicine, along with clarithromycin and azithromycin, which is recognized for its prolonged effects. Due to the allergic responses that
macrolide residues might produce because of their metabolites, consumers have an elevated risk. Another macrolide, tylosin, is used by beekeepers worldwide to treat American foulbrood, but it is not recommended as a preventative measure in healthy colonies. Tylosin usage must be stopped at least 4 weeks before honey is extracted [30, 53, 55].

Sulfonamides are among the most frequently utilized medications in veterinary medicine due to their low cost, extended antibacterial spectrum, and acknowledged therapeutic efficacy in various infectious diseases. Sulfonamides are frequently used in beekeeping to treat nosemosis and European and American foulbrood [55]. Consuming honey with sulfonamide residues might be dangerous for human health. Anaphylaxis, urticaria, and pruritus are adverse responses to food sulfonamide residues. Studies on rats revealed that the sulfamethazine in honey induces tumors with various localizations, and it is highly toxic to the thyroid gland. The most frequently utilized sulfonamides in veterinary medicine, including beekeeping, are sulfadimethoxine, sulfamethoxazole, sulfaquinoxaline, and sulfadiazines. Honey can get contaminated with these residues if the period between treatments of the bees and honey extraction is not recommended [74]. Sulfonamides have a moderate toxicological profile, which includes gastrointestinal problems (nausea, vomiting, and diarrhea), hypersensitivity (allergic) reactions that include skin rashes, eosinophilia, rarely anaphylactic shock, and the possibility of developing hemolytic anemia in individuals who have a genetic deficiency in glucose-6-phosphate dehydrogenase. Sulfonamides should not be given to infants or pregnant women in the third trimester since they compete with bilirubin for the same binding sites. Unbound bilirubin can accumulate in the subthalamus and basal ganglia of the brain, leading to toxic encephalopathy.

Moreover, sulfonamides have been linked to liver toxicity (including necrosis), drug fever, serum sickness, and systemic lupus erythematosus (type III hypersensitivity mediated by immunoglobulin G) [67, 72, 75, 76]. In addition to dermatological toxicity, sulfonamides can also cause severe allergic responses and hepatic and hematological destruction. Sulfonamides can cause a variety of allergic reactions (hypersensitivity), ranging from modest skin rashes to severe or occasionally life-threatening reactions, including erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis [77].

Quinolones are a group of synthetic antimicrobial medications that treat various infectious disorders caused by bacteria in both human and veterinary medicine [57]. They take effect by decreasing the activity of the enzymes DNA gyrase and topoisomerase IV. The determination of residues in honey demonstrated the presence of enrofloxacin, ciprofloxacin, and norfloxacin. Because they can trigger allergic reactions or the development of drug resistance in humans, quinolones used in veterinary medicine may pose a risk to human health [65, 71, 78].

Cephalosporins are a subclass of beta-lactam antibiotics that beekeepers frequently use to treat Gram-negative bacteria. Ceftriaxone, a third-generation cephalosporin exclusively utilized in veterinary medicine, has a bactericidal action by inhibiting the growth of bacteria’s cell walls. Even though it has been recommended not to be used in beekeeping in recent decades, it can still be identified in honey [53, 55, 68].

Tetracycline, oxytetracycline, dimethyl-chlortetracycline, doxycycline, minocycline, and Vibramycin are standard antibacterial chemotherapeutics used in veterinary medicine due to their broad bacteriostatic spectrum. Research has demonstrated that utilizing tetracycline in powder form leads to smaller residues than in liquid form. The FDA in the United States has authorized a variety of drugs, including oxytetracycline, to be utilized in beekeeping. But, at least six weeks before honey is extracted, its administration should be stopped [56, 65, 69].

The EU legislation has not yet established a minimum performance limit for antibiotics except for chloramphenicol (0.3 μg·kg⁻¹). Additionally, Belgium has imposed restricted levels for several antibiotics in honey, including tetracyclines and the total amount of sulfonamides (20 μg·kg⁻¹). The United Kingdom has regulated the total amount of sulfonamides as low as 50 μg·kg⁻¹, Switzerland has set it as low as 20 μg·kg⁻¹, and France has set it as low as 15 μg·kg⁻¹. Italy established a detection limit of approx. 1.5 μg·kg⁻¹ for aminoglycosides and 5 μg·kg⁻¹ for tetracyclines, sulfonamides, and macrolides [30].

The most widely used techniques for identifying antibiotics in bee honey involve screening tests (microbiological and immunological enzyme testing) and confirmatory approaches (mass spectrometric detection and chromatographic methods). Rapid tests are generally quick and cheap, but they can potentially provide false positive results. The Biochip immunochemical method has lately been compared to the LC-MS/MS method, and the results show selectivity and accuracy for quantifying drug residues in honey at very low levels [70]. Due to the uncontrolled usage of drugs, those analysis results can contribute to a greater understanding of environmental concerns and should impose preventative measures [30].

5.3. Microorganism. Bacteria, molds, and yeasts can be found in honey and honeycomb and come from bees, nectar, or other external sources such as dust, pollen, humans, tools, containers, equipment, and wind [53].

The first source of the microorganisms located in the bees’ intestines might have originated from pollen. Bee’s intestinal microbiota contains 27% Gram-positive bacteria (including Bacillus, Bacteridium, Clostridium, and Streptococcus spp.), 70% Gram-negative bacteria (including Citrobacter, Enterobacter, Escherichia coli, Klebsiella, Proteus, and Pseudomonas), and 1% yeast [53, 59].
Due to its antibacterial properties, most bacteria and microorganisms will not grow or reproduce in honey. Furthermore, honey has a relatively small amount of water, which limits bacterial development and survival. Honey has not been associated with many infections; thus, the high amount of vegetative bacteria may only result from recent contamination [64].

Many countries have reported finding *Clostridium botulinum* type F spores in various honey product containers. Nevertheless, there was no distinction between contaminated honey and sterilized honey regarding pH, HMF concentration, or diastase activity. *Bacillus alvei* may have influenced the growth of *C. botulinum* in honey because it stimulated the production of toxins by *C. botulinum* type F [53, 64, 79].

In countries like Argentina, 1.12% of samples from rural producers were found to be contaminated with *C. botulinum* type A, while 7% of samples from producers in Brazil were found to be contaminated with *C. botulinum* type A, B, and D. In the United States, 10% of samples are contaminated with the same bacteria. In Japan, 5% of samples were contaminated with *C. botulinum*, while in Finland, 8% of samples from local sources and 12% from imported honey were contaminated similarly [53, 64].

Ingestion of *C. botulinum* spores has been related to infantile botulism, the most prevalent type of disease in children. Spores ingested by newborns and children proliferate in the digestive tract and release botulinum toxin. In 15% of infant botulism cases reported to the Centers for Disease Control and Prevention, honey consumption was associated. Gamma radiation is an effective way to sterilize *C. botulinum* in honey because it stimulated the production of toxins by *C. botulinum* type F [53, 64, 79].

*Candida parapsilosis*, *Rhodotorula mucilaginosa*, *Meyerozyma caribbica*, *Occultifur externus*, *Vishniacozyma victoriae*, and *Aureobasidium pullulans* are a few yeast species related to *A. mellifera* that have been identified in the United States and Brazilian environments. *Aureobasidium pullulans* was the most prevalent on the body surface of nurse bees and forager bees in Brazil. *Aureobasidium pullulans* is a ubiquitous species that lives on the surface of fresh fruit, in water, and soil. *Rhodotorula* sp. and *Candida* sp. are frequently identified on leaves, flowers, and fruit. The genus *Candida* was linked to bees in the form of 4 species: *C. hawaiiana*, *C. oleophila*, *C. parapsilosis*, and *C. orthopsilosis*, the last being the most predominantly reported. The ubiquitous yeast *Rhodotorula mucilaginosa* has been found in various natural habitats, including flower pollen, nectar, decaying plant material, insects, and stingless bees [59].

The conventional approaches to identifying microorganisms isolated from food are focused on phenotypic techniques, including observing the morphology and growth of microbes on media, Gram staining of cells, carrying out catalase and oxidase tests, microscopically evaluating cell morphology and the type of hemolysis, and carrying out biochemical tests such as tests for indole, urease, or aminopeptidase. However, those procedures need a spectrometer operator to analyze data, which is time-consuming and highly laborious [72].

Other methods for identifying microorganisms in bee honey include immunological tests (antibody-based assays) such as enzyme-linked immunosorbent assay (ELISA) and molecular methods together with bioinformatic tools such as polymerase chain reaction (PCR), polymerase chain reaction based on repetitive sequences (rep-PCR), random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), DNA fingerprinting techniques, intron splice site priming, 16S rRNA gene sequencing, PCR in combination with sequencing, real-time PCR (quantitative PCR, qPCR), denaturing gradient gel electrophoresis (DGGE), PCR-DGGE fingerprinting, time-temperature gradient gel electrophoresis (TTGE), temperature gradient gel electrophoresis PCR (PCR-TGGE), multilocus sequence analysis (MLSA), Fourier transform infrared spectroscopy (FT-IR), and pyrolysis mass spectrometry (PyMS). Although those techniques provide accurate identification, most are complex, laborious, expensive, and time-consuming. Because of that, it is challenging to perform routine analysis utilizing any of those techniques [80].

To identify microorganisms, it is necessary to develop new techniques that are fast, accurate, reliable, highly specific, uniform for the analysis of various microorganism groups, accessible, and simple to use [80].

In that sense, proteomic methods appear to be advantageous. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) is a chemotaxonomic technique that involves the analysis of ribosomal proteins characteristic of a certain family, genus, species, or even a strain of microorganism [26, 80, 81]. Due to its simplicity of use, extremely high sensitivity, accuracy, reproducibility, and low cost, the MALDI-TOF method is becoming more and more attractive for use in microbiological diagnostics [80, 82, 83]. Using MALDI-TOF MS and 16S rDNA, comparative investigations were conducted to identify the microorganisms present in several varieties of honey from various geographical and botanical sources (such as honeydew honey, multiflower honey, and sunflower honey) [58, 80, 82]. *Bacillus spp.*, *Micrococcus sp.*, *Staphylococcus* spp., and *Lysinibacillus* spp. in honey samples were all confirmed by the results of both study methods. The MALDI-TOF method, in contrast to the 16S rDNA methodology, enabled a clear differentiation between species such as *B. subtilis* and *Bacillus cereus*, a significant discovery for study because those two species are the most frequently discovered in honey [80].

The presence of those species in bee substrates suggests that bees serve as vectors, spreading the yeast throughout the environment and carrying it into the substrates of the colony [59].

### 5.4. Heavy Metals

Heavy metals in honey refer to the presence of metallic elements with high atomic weights that exceed the acceptable limits in the honey. Those metals can contaminate honey through various sources, including polluted soil, water, air, and industrial activities. The
magnitude of honey contamination with heavy metals is substantially associated with the degree of environmental pollution, as shown in Figure 3. As a result, in highly populated and industrial areas, all bee products seemed to have concentrations over the acceptable limits for most examined metals. One indication for identifying the extent of environmental contamination is the detection of heavy metals in samples of honey [60]. On the other hand, phytosanitary treatments are one of the major issues contributing to the rise in bee family mortality nowadays [84].

It is necessary to fully understand those factors since heavy metals enter the food chain through soil, plants, and animals. Since heavy metals persist and accumulate over time, becoming a danger to both human health and the ecosystem, their discharge into the environment in large amounts has multiple consequences. Uncontrollable exposure to heavy metals has mutagenic and carcinogenic tendencies that can cause irreversible effects. Some of those consequences can even be transferred from mother to fetus [84]. Even in instances with limited exposure to heavy metals, children are susceptible to neurological problems such as attention, memory, and cognitive deficiencies [85].

Heavy metals impact all organs in the environment and in foods [86].

According to investigations, heavy metals including aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), nickel (Ni), vanadium (V), and zinc (Zn) may also be detected in honey. They can be divided into many classes based on how carcinogenic they are. As a result, they were classified into four groups: group 1—carcinogenic (Al, As, Cd, Cr, and Ni), group 2A—probably carcinogenic (Pb), group 2B—possibly carcinogenic (Co, Hg, and V), group 3—carcinogenicity not classifiable (Cr, Cu, and Se), and group 4—probably not carcinogenic (Ag, Mg, and Zn), by the International Agency for Research on Cancer (IARC) [60]. Concerns about the impact on ecosystems of increasingly high concentrations from anthropogenic sources (e.g., fuel combustion, mining, industrial industries, agricultural wastewater, and solid waste) have been highlighted [62]. The amount of heavy metals in the body and their toxicological consequences are influenced by many components, including metal intake or inhalation, metal entry rate, tissue distribution, concentration attained, and metal excretion rate [62].

Enzyme inhibition, protein synthesis inhibition, nucleic acid function variations, and cell membrane permeability alterations are just a few of the mechanisms of toxicity. Hepatotoxicity, nephrotoxicity, and neurotoxicity are some significant consequences of heavy metals [60].

Heavy metal concentration in honey varies from 0.02 to 1.03 g/100 g [6, 61, 84, 87]. Various techniques, including atomic absorption spectroscopy (AAS), atomic emission spectroscopy (ICP-AES), total reflection X-ray fluorescence (TXRF), inductively coupled plasma spectroscopy (ICP-AES), and spectrometry (ICP-OES), have been utilized to measure the concentration of heavy metals in honey, in the last two decades. One of the most widespread environmental pollutants is Pb. Lead contamination in honey can occur due to human activities such as industrial emissions, lead-based pesticides, or lead-containing paints. Ingesting honey contaminated with lead can be particularly harmful, as lead is a neurotoxic metal that affects the nervous system and brain development, especially in children [60, 87].

Cd is one of the heavy metals recognized as bioindicators for contaminated honey and lead. Cadmium contamination often results from industrial activities, waste incineration, wastewater, and fertilizers. Long-term consumption of honey contaminated with cadmium can lead to kidney disease and increase the risk of various health problems, including bone disorders [60, 88].

Fe is another metal found in honey from anthropogenic sources. Drilling, digging, and metal corrosion constitute the most common sources of Fe [60, 61, 88].

Even in extremely low quantities, public health is severely threatened by Hg, one of the most hazardous heavy metals. The agricultural area surrounding the hive, mines, combustion, and industrial and municipal sewage systems are the sources of Hg toxicity. Mercury is a potent neurotoxin and can cause severe neurological issues, particularly in developing fetuses and young children [60, 89].

Nevertheless, given its high acidity, bee honey can also corrode stainless steel and/or galvanized steel containers, releasing heavy metals such as Pb, Cr, Al, Ni, and Sn during harvesting, processing, preparation, and storage [60].

Based on the formula THQ = EDI/RfD, the carcinogenic risk related to the presence of heavy metals in honey is estimated. THQ refers to the target hazard quotient, where EDI is the dose of heavy metals ingested (μg/kg-d), and RfD or TDI is the oral reference dosage (mg/kg-d) [60, 90]. THQ values greater than 1 are harmful to human health [91]. Following the THQ value, Pb is the most dangerous element, followed by Cd, Mn, Fe, Ni, As, Cu, Hg, and Cr. THQ levels
in honey have reportedly increased in Turkey, Iran, Bulgaria, Bangladesh, Poland, Austria, Mexico, Italy, Nigeria, France, Argentina, Pakistan, Spain, United States, Brazil, Kosovo, Canada, Australia, Switzerland, Germany, Greece, China, Slovakia, Lithuania, Bosnia, Herzegovina, Algeria, and Macedonia, among other countries. Consequently, consumers in Turkey, Iran, and Bulgaria seem more vulnerable to illness than those in other countries [3, 60, 91]. Thus, beekeepers must regularly check the soil in agricultural areas and agriculturally utilized waterways and avoid placing beehives in places with a lot of industrial activity to prevent the buildup of contaminants in their products [60].

Regulatory authorities in different countries have established maximum permissible limits for heavy metals in food, including honey, to safeguard public health. Monitoring and controlling heavy metal levels in honey are essential to ensure its safety for consumption. Regular testing of honey for heavy metal contaminants and enforcing stringent quality control measures in beekeeping practices and honey processing can help prevent heavy metal contamination.

Consumers can also play a role in reducing the risk of heavy metal exposure from honey by purchasing products from reputable sources with proper labeling and certifications. Being aware of potential environmental sources of heavy metals and supporting sustainable practices that reduce pollution can also minimize heavy metal contamination in honey and protect our health.

6. Final Considerations

The desirable physical, chemical, sensory, and healing properties of bee honey have positioned it as an appealing and commercially viable functional food. It also emphasizes the dangers of consuming honey that has been tampered with or contaminated by pesticides, antibiotics, microorganisms, and heavy metals. Bee honey plays a significant role in assessing environmental pollution, acting as a mirror of the current environmental condition. Consuming adulterated and contaminated honey threatens safety, food security, and environmental sustainability. Contaminated honey can lead to genetic abnormalities, allergic reactions, and carcinogenic effects while also contributing to the development of antibiotic resistance in certain microorganisms. Various analytical techniques have been employed over time to identify residues in honey. Unfortunately, there is currently no established regulation determining the minimum acceptable levels of contaminants for honey.

In light of those concerns, honey producers and processors must be well-informed about honey contaminants and adhere to the regulations that ensure the safety and authenticity of their honey products. Furthermore, consumers can make informed decisions by scrutinizing product labels for pertinent information and seeking certifications that confirm adherence to quality and safety standards. It is worth noticing that the specific laws and regulatory bodies governing honey may vary from country to country, highlighting the importance of consulting the relevant local or national authorities for the most current information.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

L.A., M.V., V.V.L., I., B.-A.M., A.L., P.-C.M., O.-L.P., V.M.B., I.M.S., and L.T. contributed equally with I.-D.M. to this article. All authors have read and agreed to the published version of the manuscript.

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