Antibiotics, pesticides, and heavy metals contaminants of honey as affected by antibiotics usage and agricultural practices in different Egyptian environments

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Background and aim
Environmental pollutants and global climate changes have a negative health effect on honeybees, and increase honey contamination.The aim of this study was to test the effect of antibiotic usage and agricultural practices on the presence of a total of 461 pesticides, 30 antibiotics, and five elements traces in honey samples collected from Egyptian apiaries of different environmental conditions representing intensive, and limited agriculture production regions.
Materials and methods
Pesticides and antibiotic residues in honey were detected at trace levels using tandem mass spectrometry techniques Gas Chromatography-Mass Spectrometry/Mass Spectrometry and Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (GC-MS/MS and LC-MS/MS).
Antibiotics were detected using only Liquid Chromatography-Mass Spectrometry/Mass Spectrometry LC-MS/MS. The quadrupole inductively coupled plasma mass spectrometry technique (QICP-MS) was applied for the trace element analysis.
Results and conclusion
Amitraz and acetamiprid were more frequent. The highest concentration of amitraz (0.022 mg/kg) was found in samples obtained from apiaries in the north delta. Iron and zinc were the highest frequently detected elements in all the collected honey samples. Also, Cu was less frequently detected elements in honey samples with percent values of 7%. Cd and Pb were found in honey samples from apiaries in the south delta of Egypt at 20%, and 27%, respectively. Most of the collected samples were contaminated with antibiotics. A direct relation between agriculture production and uncontrolled antibiotics applications on a beehive was concluded due to the increased diseases of bees in the regions of intensive agriculture production. Only two pesticides were detected along with low concentrations of toxic elements in too low levels to exceed their 'European Union Maximum Residue Limit' EU MRL.

Keywords:
Antibiotics, apiaries, honey contamination, mass spectrometry, pesticide residues

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Introduction

Honey is a popular natural rich food. Honey production is well known and accomplished from the nectar of plants that are subsequently combined with specific bee secretions, physically dehydrated, and finally stored in honeycombs [1]. In developing countries, honey production is not only a popular rich food but also a source of financial support for several families and small stakeholders [2]. Honey is susceptible to chemical contamination from various sources, including environmental factors and beekeeping practices. Its production is directly related to the amount of flora in the surrounding agricultural area. Worldwide pesticide usage increases in crop production [2], resulted in an increase in the probability of the accumulation of pesticide residues in honey, especially in developing countries where pesticide practices are not well controlled [3,4]. These residues deteriorate honeybees' immune system and result in more disease and death reports [5]. The presence of tau-fluvalinate was reported in the Egyptian honey [6] and beeswax [7]. Also, several trace elements can be found in honey as a result of environmental geographic compositions, various industrial contaminations, and uncontrolled agricultural practices such as the excessive usage of fertilizers and pesticides [8,9].

Honey bees (*Apis mellifera* L.) are susceptible to several infections and diseases such as varroosis which caused by *Varroa spp. (Varroa destructor)*, small hive beetle (*Aethina tumida*), American foulbrood (*Paenibacillus larvae*), and *Tropilaelaps spp* [10]. In addition, Egyptian honey bees are also susceptible to nosemosis, the historical well known *Nosema apis*, instead of *Aethina tumida* [11]. Therefore, Antibiotics such as tetracycline, streptomycin, chloramphenicol, and moroxydine were used for treatment [12,13]. Large losses of honeybee colonies were reported in intensive agriculture production areas [14]. Intensive and uncontrolled agriculture habitats are characterized by uncontrolled pesticide practices. Under these conditions, honeybee diseases increased through pathogens transmissions with other different living species that share the same flowers. Such transmitted diseases are two-fold with the regular movement of the bee hives between different crops and during the international uncontrolled shipping [15]. A negative synergetic health impact of parasites and pesticides on the immune-competence of honey bees was also reported [16]. In addition, environmental pollutants and global climate changes have a negative health effect on honeybees, increasing honeybees' diseases with minimizing the efficacy of used antibiotics [17]. Such circumstances resulted in negative health effects on honey bees, subsequent increased antibiotic application, and finally contamination of honey by elevated antibiotic concentrations especially during uncontrolled agricultural practices. It was reported that ciprofloxacin and oxytetracycline were detected at high residue concentrations in Egyptian honey samples [18]. In Korean honey, chlortetracycline and tetracycline were found to be the most frequently detected antibiotics [19], while sulphonamides were the most frequently detected antibiotic in Italian honey [20].

Gas Chromatography-Mass Spectrometry/Mass Spectrometry and Liquid Chromatography-Mass Spectrometry/Mass Spectrometry techniques (GC-MS/MS and LC-MS/MS) are used to analyze both polar and nonpolar pesticides at trace levels, but only Liquid Chromatography-Mass Spectrometry/Mass Spectrometry LC-MS/MS is used to identify antibiotic residues in honey [21,22]. For the analysis of trace elements, the quadrupole inductively coupled plasma mass spectrometry technique (QICP-MS) has been used.

The study aimed to investigate the impact of agricultural practices and antibiotic usage on the presence of traces of 30 antibiotics, five elements, and 461 pesticides in honey samples collected from Egyptian apiaries in varying environmental conditions representing limited (Sinai and New Valley) and intensive (North and South Delta) agriculture production regions. Both gas and liquid tandem mass spectrometry techniques (GC-MS/MS and LC-MS/MS) were used to analyze the traces of both polar and nonpolar pesticides, and LC-MS/MS to detect antibiotics traces.

Materials and methods

Sampling

In 2022 and 2023, a total of 60 samples of honey were taken from apiaries that are bordered by areas of intensive and limited agricultural production. Thirty samples were collected from apiaries in the North and South Delta, representing the intensive agriculture production regions in Egypt. The rest of the samples were collected from Sinai and the New Valley (representing limited agriculture production regions in Egypt). The collected apiary honey samples were directly taken from the sealed honey inside the beehive, then filtered using a fine mesh screen, collected into a sterilized glass jar, and finally kept at room temperature for analyses.

Chemicals and reagents

Methanol and formic acid were obtained from Merck-Millipore and Fisher-Scientific, respectively. Acetonitrile and disodium ethylene diamine tetra acetic acid (Na_2EDTA) were purchased from CARLO ERBA and Fluka, respectively. Ready-prepared QuEChRS salt mixtures for the salting out (4.0 g anhydrous magnesium sulfate, 1.0 g tri hydrogen sodium citrate, 0.5 g di hydrogen sodium citrate, and 1.0 g sodium chloride). For the sample clean-up, 1.0 g anhydrous magnesium sulfate and 0.2 g primary secondary amine (PSA) were used (Agilent Technologies, USA). Concentrated nitric acid (65%, w/w) and hydrogen peroxide (30%, w/w) were purchased from Merck-Millipore. Sodium hydroxide and citric acid monohydrate were purchased from Riedel deHaen. Deionized water (DIW), of >17.5 Ω cm, was prepared using a Millipore water purification system (Milli-Q).

Pesticide residue analysis

Standard QuEChRS methodology was utilised to obtain the pesticide residues from the honey samples that were collected in the Central Laboratory for Analysis of Pesticide Residues and Heavy Metals in Food (QCAP Lab).

Standard solutions

Pesticide reference standards were obtained from Dr. Ehrenstorfer GmbH in Augsburg, Germany. To prepare a single stock standard solution, 1000 µg/ml, 10 ml of toluene was used, and it was kept at −22°C. Several effective pesticide solution combinations were made in methanol at varying concentrations, and they were kept cold at −4°C.

Sample preparation

A modified version of the QuEChERS protocol was used [6]. To sum up, 50 ml polypropylene centrifuge tubes were filled with 5.0±0.02 g of honey, 10.0 ml of DIW, and combined with a vortex. There after kept the sample submerged in a water bath at 40°C until it was completely homogenised. A mechanical vertical shaker was used to add and shake 10 ml of acetonitrile for a duration of one minute. After adding the ready-made salting-out mixture, the mixture was quickly agitated for one minute and centrifuged for five minutes at 15,000 *g*, 4–8°C. Six milliliters of the acetonitrile fraction were put into a 15 milliliter d-SPE polypropylene tube, agitated for a minute, and then centrifuged at 15,000×*g* for two minutes. For LC-MS/MS analysis, an aliquot was directly collected using a 0.45 um PTFE syringe filter and placed into a glass vial. A glass vial was filled with an aliquot that was filtered using a 0.45 um PTFE syringe for the GC-MS/MS analysis after 2.00 ml of the supernatant was transferred into a 50 ml round bottom glass flask, evaporated, and exchanged using the same volume of hexane/acetone (9 : 1, v/v) containing 100.0 µg/l aldrin, as an internal injection standard.

Mass spectrometry analysis

Pesticide residue analyses in honey were done using both LC-MS/MS and GC-MS/MS to enable the detection of a wide scope of pesticides ranging from highly polar to highly nonpolar pesticides.

LC-MS/MS

Chromatographic separation of the tested pesticides was conducted using an Agilent HPLC system (1200 Series) and a C18 column (Poroshell 120 EC-C18; 50 mm, 4.6 mm, and 2.7 µm). The used mobile phase includes solvent (A); 10 mM ammonium format in methanol-water solution (1:9, v/v) and solvent (B); 100% methanol. The elution program of these pesticides was applied as previously reported [7]. The column oven temperature was adjusted at 40°C, injection volume of 2 µl, and a constant flow rate of 0.4 ml/min. LC-MS/MS 6500^{+}(AB-SCIEX) was applied using the following mass parameters; ion spray voltage of 5000 V, entrance potential of 10 V, and a temperature of 450°C. The used gases in the ion source were 50 PSI [nebulizer gas (gas 1) and heating gas (gas 2)], in addition to the curtain gas (25 PSI). The multiple mass reaction monitoring (MRMS) and related collision energies were as previously reported [23].

GC-MS/MS

The used GC–MS/MS was an Agilent 7890B gas chromatograph and 7010B Mass Spectrometer with electron ionization mode. The pesticide chromatographic separation was achieved using an HP-5 MS ultra-inert column (30 m×0.25 mm, 0.25 µm, Agilent). A highly pure helium gas (>99.999%) was used as carrier gas with a flow rate of 1.83 ml/ min. As was previously reported, oven temperature programs and MRM transitions were also used in this study [7,24].

Antibiotic residue analysis

Antibiotic residue analyses in the collected honey samples were done using the previously reported by Ryad *et al.* [18]. Analyses were done in the Central Laboratory for the analysis of pesticide residues and heavy metals in food (QCAP Lab).

Sample preparation

Two-grams honey sample ±0.02 g was weighed into 50 ml polypropylene centrifuge tubes and dissolved using 1 ml sodium citrate buffer solution (1 M, pH 4). After adding 0.5 ml of di-sodium ethylene diamine tetra acetic acid to the centrifuge tubes, they were placed on an ultrasonic shaker at 50°C for 30 min. A volume of 10 ml acetonitrile was added and shaken for 10 min. The sample was centrifuged for 10 min at 15,000×*g* and 4°C. The resulting supernatant was collected in a 100 ml round bottom glass flask. Another 10 ml acetonitrile was added to the remaining honey sample then shaken for 1 min, and centrifuged for 10 min at 15,000×g and 4°C. The resulting supernatant was collected also into the previous bottom glass flask. Finally, the sample was evaporated to dryness at 35°C.

Standard solutions

One stock standard solution for each antibiotic of 1000 µg/ml concentration, was prepared in 10 ml methanol and stored at −20°C. A working antibiotic solution mixture of different concentrations was prepared in methanol and stored at −4°C.

Mass spectrometry analysis using LC-MS/MS

Details of chromatographic separation and mass parameters of the detection of the tested veterinary

drugs were reported by Ryad *et al.* [18]. Briefly, the used LC-MS/MS was a Sciex API 4000 Triple Quad system with Agilent HPLC model 1200. The HPLC separation was performed using a C-18 column (Zorbax, 2.1 mm×50 mm, 1.8 µm). Ionization was performed using electrospray ionization (ESI) in both positive and negative modes using a source potential of 5500 V.

Trace toxic elements analysis

The analysis of lead (Pb), cadmium (Cd), and other trace elements including iron (Fe), copper (Cu), and zinc (Zn), in the collected honey samples were done using the previously reported test by Ghunium *et al.* [25], in the Central Laboratory for analysis of pesticide residues and heavy metals in food (QCAP Lab).

Standard solution

Certified reference stock standard (1000 mg/l, 2% HNO_3 solution) of the tested elements were purchased from Merck.

Sample preparation

Details of the sample preparation were performed as reported by *Ghuniem et al* [25]. I, Brief, a honey sample portion of 0.5 g was weighed into a microwave digestion vessel. Suprapur nitric acid (8 ml) was added, followed by a gentle shaking, then 2 ml of hydrogen peroxide was added. The microwave oven program of 1800 W was applied for 15 min until the temperature reached 200°C. After the temperature was hold for 15 min, the microwave was vented until a temperature became below 80°C. The thermocouple probe was removed from the reference vessel and the vessel was cooled down in a water bath for a period of 30 min. The lid and walls were rinsed down with deionized water inside the vessel, then the residual solution was transferred to a 50 ml polymethyl pentene volumetric flask. The internal standard mixture of Bismuth (Bi), Germanium (Ge), Indium (In), Lithium-6 isotope (6Li), Scandium (Sc), Terbium (Tb), and Yttrium (Y) (0.5 ml) was added by filling the flask using DIW. A sample portion was kept in a polypropylene tube for the Q-ICP-MS measurements.

Instrumental

Details of the instrumental conditions were performed as reported by Ghuniem et al [25]. Briefly, the vacuum and water cooler of the QICP-MS were turned on before the plasma ignition, at least 30 min before starting instrument optimization. The Q-ICP-MS analysis was performed using kinetic energy discrimination (KED) mode. Helium gas is used inside the collision cell. The helium gas flow rate is 1 ml/min for the measurement of Pb, Cd, and Cu. The flow rate was 4 ml/min for the measurement of Fe, and 4.6 ml/min for the measurement of Zn. The ICP radio frequency power is equal to 1600 W (analog to −1800 V), the pulse stage voltage is equal to 1000 V, the deflector voltage is equal to-10.25 V, the cell entrance voltage is equal to −6 V, and the cell exit voltage equal of −39 V.

Statistical analysis

Standard deviations (S.D) and mean values for every group were computed as part of descriptive statistics. One-way analysis of variance was utilised to evaluate statistical differences among means (ANOVA). The Least Significant Difference (LSD) test was used to separate means at the 5% probability level when significant differences were found.

Results

Pesticide residues in honey

Analysis of variance and mean separation tests indicated significant differences among samples collected from apiaries of different environmental conditions representing intensive and limited agriculture production regions during 2022 and 2023 in pesticide residues and trace elements concentrations. Only two out of the 461 tested pesticides in honey samples were detected (Table 1). These are the insecticides Amitraz and Acetamiprid. Only samples from Sinai were free of pesticide residues. Amitraz was the most frequently identified pesticide, and was present in 27% of the samples, while Acetamiprid was found in 7% only.

Trace element in honey samples

Main values and standard errors of Fe, Cu, Cd, Pb, and Zn in honey samples are presented in Table 1. Iron and zinc were the highest frequently detected elements in all the collected honey samples with values of 100% in New Valley, and 87% in Sinai. The highest mean concentrations of Fe were 10.73 mg/kg, and Zn was 6.70 mg/kg, respectively detected in the New Valley. Honey samples from apiaries in the New Valley have the highest concentrations of Fe and Zn which may be attributed to the high elemental composition of these metals in this geographic area. The detected Fe in all the collected honey samples ranged from 1.9 to 27.8 mg/kg (Table 1). On the other hand, Cd and Pb were highly frequently detected in honey samples at 20%, and 27%, respectively, from apiaries in the south delta of Egypt.

These elements were detected with concentrations below the limit of quantifications but gives an

Table 1 Pesticide residues, trace elements concentrations (mg/kg) and their frequency (%) in the honey samples collected from Egyptian apiaries of different environmental conditions representing intensive and limited agriculture production regions during 2022 and 2023

	Fe	Zn	Cd	Cu	Pb	Amitraz	Acetamiprid
Sinai							
Conc. Range	2-8	1-18	n.d.	n.d.	n.d.	n.d.	0.02
Det. Freq	87%	87%	n.d.	n.d.	n.d.	n.d.	7%
Mean	4.35^{bcd}	5.11^{b}	0.00	0.00	0.00	0.00	0.001^{ab}
S.D	1.69	5.36	0.00	0.00	0.00	0.00	0.006
S.E	0.44	1.38	0.00	0.00	0.00	0.00	0.002
South Delta							
Conc. Range	3-16	1-22	>LOQ	1	>LOQ	0.01-0.02	0.04
Det. Freq	93%	60%	20%	7%	27%	13%	7%
Mean	6.64^{b}	2.91^{d}	0.00	0.08^{b}	0.00	0.002^{ac}	0.003^{a}
S.D	3.10	5.32	0.00	0.23	0.00	0.005	0.009
S.E	0.80	1.37	0.00	0.06	0.00	0.001	0.003
North Delta							
Conc. Range	2-13	1-19	>LOQ	n.d.	n.d.	0.01-0.02	n.d.
Det. Freq	73%	80%	7%	n.d.	n.d.	27%	n.d.
Mean	6.31^{bc}	3.71^{c}	0.00	0.00	0.00	0.006^{a}	0.00
S.D	3.20	4.43	0.00	0.00	0.00	0.007	0.00
S.E	0.82	1.14	0.00	0.00	0.00	0.001	0.00
New Valley							
Conc. Range	2-28	1-60	n.d.	4	>LOQ	0.01	0.01
Det. Freq	100%	73%	n.d.	7%	13%	13%	7%
Mean	10.73^{a}	6.70^{a}	0.00	0.29^{a}	0.00	0.002^{ab}	0.0007^{abc}
S.D	7.45	15.54	0.00	1.14	0.00	0.004	0.002
S.E	1.92	4.01	0.00	0.29	0.00	0.001	0.0007
P	0.002	0.66	0.00	0.47	0.00	0.01	0.68
LSD	3.23	6.53	0.00	0.42	0.00	0.004	0.004

$^{a-d}$Different letters in the same column indicate significant differences among locations in residues concentration ($P<0.05$). Conc. range, concentration range; Det. freq., detection frequency; LOQ, limit of quantification; n.d., not detected; S.D, standard deviation; S.E, standard error.

indicator for the existence of environmental contamination in the intensive agriculture production area (Table 1). Elements such as Ni and Cr were undetectable in the honey samples.

Antibiotics residue analysis in honey samples

Results indicated that only the collected honey samples from Sinai were free from any residue of the tested 30 veterinary antibiotics. Most of the collected samples from the rest of the investigated regions were contaminated by antibiotics, especially in the North and South Delta. Only seven antibiotics (ciprofloxacin, doxycycline, sulfadiazine, sulfamethoxazole, sulfamethazine, trimethoprim, and Tylosin) were detected in different concentrations (Table 2). The differences among the locations were statistically significant in terms of sulfamethoxazole, and trimethoprim, ($P<0.05$). Tylosin was detected with the highest concentrations in all the tested samples of concentrations equal to 7780, 4460, and 1434 μg/kg in the South Delta, New Valley, and North Delta regions, respectively. It was observed that most of the antibiotic-contaminated samples from the north and the south delta were simultaneously contaminated by at least two to five antibiotics. Few samples from the new valley were simultaneously contaminated by more than two antibiotics (Table 2). Trimethoprim and sulfamethoxazole antibiotics had the highest detection frequency. Trimethoprim frequency was 73%, 53%, and 47% while sulfamethoxazole frequency was 67, 53, and 40% in the collected honey samples from North Delta, South Delta, and New Valley, respectively.

Discussion

Amitraz was the most frequently identified pesticide, and was present in 27% of the samples, while acetamiprid was found in 7% only. This may be attributed to the direct application of amitraz in beehives as an acaricide to control various honeybee diseases, particularly *Varroa destructor*, vectors of dangerous viruses such as the Deformed Wing Virus (DWV) and the Acute Bee Paralysis Virus (ABPV) [26]. Nevertheless, exposure to amitraz may negatively impact bees' ability to fend against viral infections [27]. Furthermore, a number of honeybee physiological processes, such as heart rate, detoxification,

Table 2 Antibiotics residues concentrations (μg/kg) and their frequency (%) in the honey samples collected from Egyptian apiaries of different environmental conditions representing intensive and limited agriculture production regions during 2022 and 2023

	Sulfadiazine	Sulfamethoxazole	Trimethoprim	Tylosin	Ciprofloxacin	Sulfamethazine	Doxycycline
Sinai							
Conc. range	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Det. Freq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E	0.00	0.00	0.00	0.00	0.00	0.00	0.00
South Delta							
Conc. range	10-483	14-687	18-600	14-7780	385	17-39	n.d.
Det. Freq	47%	53%	53%	33%	7%	13%	n.d.
Mean	76.52^{b}	80.54^{ab}	134.59^{ab}	632.73^{a}	25.63^{b}	3.71^{b}	0.00
S.D	143.85	176.27	206.07	2021.97	99.28	10.61	0.00
S.E	37.14	45.51	53.208	522.07	25.63	2.74	0.00
North Delta							
Conc. range	11-1512	17-683	45-1176	10-1434	10-45	648	n.d.
Det. Freq	47%	67%	73%	53%	13%	7%	n.d.
Mean	121.19^{a}	180.03^{a}	287.42^{a}	121.02^{b}	3.69^{c}	43.22^{a}	0.00
S.D	386.96	248.59	371.49	370.07	11.81	167.39	0.00
S.E	99.91	64.18	95.91	95.55	3.05	43.22	0.00
New Valley							
Conc. range	52-62	52-341	8-539	37-4460	19-323	n.d.	57
Det. Freq	13%	40%	47%	20%	20%	n.d.	7%
Mean	7.60^{c}	61.75^{c}	99.07^{ac}	303.74^{c}	25.93^{a}	0.00	3.79^{a}
S.D	20.14	103.21	164.11	1149.92	83.26	0.00	14.67
S.E	5.20	26.65	42.37	296.91	21.49	0.00	3.78
P	**0.33**	0.02	**0.01**	**0.49**	**0.56**	**0.42**	**0.23**
LSD	**151.17**	117.68	**166.56**	**861.44**	**47.58**	**61.34**	**16.38**

$^{a-d}$Different letters in the same column indicate significant differences among locations in antibiotics residues concentration ($P<0.05$). Conc. range, concentration range; Det. freq., detection frequency; n.d., not detected; S. E, standard error; S.D, standard deviation.

immunology, antioxidant capacity, and queen development, can be adversely affected by amitraz and its metabolites [28]. The honey samples taken from apiaries in the north delta had the highest amount of amitraz (0.022 mg/kg). Compared to the EU-maximum residue limit (EU-MRL) of 0.05 mg/kg, this value is far lower. It's possible that their low consumption concentrations prevented the degradation products of amitraz DMPF (N-(2,4-dimethylphenyl)-N0-methylformamide) and DMF (2,4-dimethylformanilide) from being found in the examined samples. While the US environmental protection agency (E.P.A) has established MRLs for amitraz (0.2 mg/kg), Comaphos (0.1 mg/kg) and Fluvalinate (0.05 mg/kg). Notably, the maximum limits of pesticides residues (LPR.) in honey are not outlined in the Codex Alimentarius (CA**)** [29]. Pesticides are worldwide used to control bee diseases and pests. Their administration is uncontrolled and applied without approval protocol. Comparison with findings from different countries demonstrates variations in pesticide residues in honey. Ninety-two pesticides, including six from toxicity class IA (extremely hazardous), eight from class IB (highly hazardous), forty-two from class II (moderately hazardous), thirty-five from class III (slightly hazardous), and one from class IV (not posing a serious threat), were found to have residues in honey from twenty-seven countries [30]. The hazard indices (HI) showed that honey posed a significant risk to one's health. In 109 honey samples taken from shops and marketplaces in Turkey, 24 organochlorine pesticide residues were found; the majority of these residues were higher than MRLs [31]. In France, a field survey was initiated in apiaries and residues of 14 pesticides and two fungicides were detected. Ebdo salfane residues were the most frequently occurring residues [32]. During 2002, it was found that Portuguese honey was more contaminated than the Spanish one [33]. However, the American honey samples from Virginia showed no comophos or fluvalinate residues exceeding the MRL limit [34]. Bogdanove [35] reviewed various studies conducted on organochlorine pesticide residues in honey, noting that the level found in different countries differed considerably. The majority of the honey samples examined from Jordan in 1995 contained residues of organochlorine pesticides, such as lindane and organophosphorus pesticides [36]. Our

findings concurred with those of Tillotson *et al.* [37], who discovered that pesticide residues in honey aid in determining the degree to which pesticides have been applied to the field crops surrounding apiaries and aid in determining the possible risk of those pesticides to human health. Also, acetamiprid (a neonicotinoid insecticide) was detected in some honey samples but at lower frequencies without surpassing its EU-MRL of 0.1 mg/kg. The highest concentration of acetamiprid (0.038 mg/kg) was found in a honey sample from Upper Egypt. Moreover, Gaweł *et al.* [38] described acetamiprid and thiacloprid concentrations in honey and found a range of 0.001 to 0.13 mg/kg and 0.001 to 0.2 mg/kg, respectively.

Honey is a good source of the major trace elements that humans need. Minerals like iron, copper, and zinc are necessary for human health. They have a significant role in several biological processes [39]. These can be toxic if their levels exceeded the safety level [40]. The trace and toxic elements analyses might be used to identify the geographic source and the degree of surrounding environmental contamination of the collected honey samples. Our data indicated that trace elements concentrations were below the upper tolerable intake level for adults [41]. Honey samples from apiaries in the New Valley have the highest concentrations of Fe and Zn which may be attributed to the high elemental composition of these metals in this geographic area. The detected Fe range in all the collected honey samples ranged from 1.9 to 27.8 mg/kg which is near the previously mentioned range of 1.8-10.2 mg/kg in Turkey [42]. On the other hand, lower than that was found in the honey samples collected from Saudi Arabia [43]. The detected concentrations of Zn in bee honey may be due to its storage and transfer using galvanized containers [8]. Also, Cu was frequently detected in the collected honey samples with values of 7% in South Delta and New Valley.

The number of elements in honey is correlated with the quantity of pollution in the bees' habitat. As per the European Communities Regulation (EC Regulation 1881/2006) [44], honey as a food product, needs to fulfil the standards for the highest possible Pb content of 0.10 mg/kg. For Cd, however, no upper limit has been set [45]. On the other hand, Cd and Pb were highly frequently detected in honey samples at 20%, and 27%, respectively from apiaries in the south delta of Egypt. Although these elements were detected with concentrations below LOQ, it gives an indicator for the existence of environmental contamination in the intensive agriculture production area. The relationship between the heavy metals contents in honey resulted from the surrounding environmental conditions (soil, water, and air) or resulted from anthropogenic activities like agricultural practices and industries. Additionally, the processing of honey using beekeepers' equipment or materials such as aluminum, stainless steel, and galvanized steel might introduce some polluting metals (Fe, Cu, Pb, and Zn) into honey [46]. Also some agriculture practices as using organic feltrizations in intensive origins to increase plant growth and crop yields are the main source of Zinc in honey. Demirezen *et al.* [47] reported that heavy metals accumulation is higher in bee products from industrial regions and areas with heavy vehicle traffic, especially those close to large settlements and garbage incinerators. Elements such as Ni and Cr were undetectable in the honey samples. This is consistent with previous research findings of Taha and Ali [48]. Conversely, honey samples from Pakistan showed the presence of such elements [49]. Furthermore, the detection of heavy metals in honey samples from Kafr El Sheikh province in Egypt revealed the presence of Fe, Zn, and Pb and that agreed with our findings [8]. The concentration of Zn in bee honey might occur during its storage and transfer into galvanized containers. Additionally, toxic elements (Cd and Pb) were only detected in a few samples, with concentrations below LOQ. The location of the apiaries, where internal combustion engines are the primary source of pollution, determined the amount of lead in the honey. Lead may also be found in the environment from car exhaust fumes. Lead can be found in soil, water, and plants, and it can eventually find its way into human diets. Lead can pollute nectar or the air [34]. The maximum permitted lead value in sweet nutrients, such sugar and honey, should be less than 0.3 μg/g, according to a comparison of the recorded heavy metal values and the pollution guidelines set by the Codex Alimentarius Commission. This constraint may have its roots in the usage of low-quality plastic containers for storing honey [50]. Also Bratu *et al.* [51] mentioned that trace elements and heavy metals could be found in higher concentration in honey found in close proximity to certain industrial areas. This analysis of trace elements in honey showed their variability, reflecting regional differences, potential environmental influences, and the impact of various processing and storage methods on the metal composition of honey samples.

Results indicated that the antibiotics Trimethoprim and sulfamethoxazole had the highest detection

frequency in honey samples from North Delta, South Delta, and New Valley, respectively. These antibiotics were also previously reported as the highest frequently detected antibiotics in collected honey samples from the north delta [13]. Five antibiotic chemicals, including tetracycline, oxytetracycline, sulfadiazine, tylosin, and chloramphenicol, were successfully isolated and found in honey samples in a survey study conducted in China [52]. After being treated in beehives, tetracycline residue was found in honey in France [53]. Honey samples from the Spanish cities of Granada and Almeria contained traces of tylosin and sulfadiamiden [54]. It was discovered that streptomycin residues affected honey in Germany [55]. While some EU nations prohibit the use of antibiotics in beekeeping, there are no MRLs set for antibiotics in honey. The selling of honey containing antibiotic residues is prohibited by European Community Regulation (E.C.R.) [56]. The action limit for antibiotics in honey is set by some nations, such as the UK, Belgium, and Switzerland, and it typically ranges from 0.01 to 0.05 mg/kg for each antibiotic group [57–59]. In another investigation, tylosin, chloramphenicol, and tetracycline were confirmed to be present in 89, 47, and 31%, respectively of the 64 honey samples examined in Egypt [60]. According to our data, it is possible to conclude that the majority of the honey samples examined, include antibiotic residues that might be regarded as contaminants. Strong regulatory measures are required to limit antibiotic usage and guarantee the safety and integrity of honey intended for human consumption, as these trends in the uncontrolled administration of antibiotics are worrisome.

Conclusion

This study focused on the primary chemical contaminants found in honey, particularly in regions marked by intensive agriculture production. While only two pesticides were detected in minimal concentrations, they were still below the EU Maximum Residue Limits (MRL). Toxic elements were found in negligible amounts and were below LOQ. The prevalence of antibiotics emerged as the predominant concern. The identification of five antibiotics present in elevated concentrations within numerous honey samples collected from regions characterized by intensive agricultural practices was a significant finding that underscores a direct correlation between agriculture production and unregulated antibiotic application in beehives as a result of the escalating diseases observed in honey bees within such regions. The presence of antibiotics in honey not only poses immediate health concerns for consumers but also raises significant long-term apprehensions, particularly regarding bacterial resistance. Addressing this issue necessitates robust management strategies that aimed at the reduction of honey bee diseases and as a result less antibiotic usage. To mitigate the risks associated with antibiotic-contaminated honey, immediate steps need to be taken including the prohibit of the sale of honey produced from unregistered apiaries, post the essential requisite tests before honey marketing, and ensure consumer safety by upholding honey quality standards. In summary, this research emphasizes the pressing need for comprehensive management practices to curtail the rising incidence of honey bee diseases, coupled with effective control measures to regulate antibiotic usage. Strict regulations in honey production and sales are imperative to safeguard consumer health and maintain the integrity of honey as a natural food product.

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Conflicts of interest

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

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