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Assessment of Heavy Metal Contamination in Honey as a Bioindicator of Environmental Pollution Associated with Oil Well Activities in Kirkuk Province, Iraq

Suzan D Hadi[1], Watban Jassim Mohammed[2], Mayson Thafir Hadi[2], Zainab A. Jabarah[3], Ali M. Saadi[4*]

[1]Department of Agricultural Mechanization and Equipment, College of Medicinal and Industrial Plants, University of Kirkuk, Kirkuk, Iraq.

[2]Department of Food Sciences, College of Agricultural Engineering Science, University of Baghdad, Baghdad, Iraq.

[3]Department of Agricultural Mechanization and Equipment, College of Agricultural Engineering Science, University of Baghdad, Baghdad, Iraq.

[4]Department of Biotechnology and Food Science, Technical Agricultural College, Northern Technical University, Mosul, Iraq

Abstract

Background: honey is produced and consumed all over the world and is regarded as one of the most significant and nourishing foods known to humanity. The habitat, type of food honey bees mainly consumes, and other related variables all influence the element content of honey. The current study aims to investigate the quantities of the following elements, such as lead (Pb), cadmium (Cd), nickel (Ni), and vanadium (V), in honey produced from hives located near oil extraction areas in Kirkuk, Iraq.
Methods: fifteen samples of honey, each with individual colony were collected from 5 different locations. Following microwave digestion, a graphite furnace atomic absorption spectrometer (GFAAS) was applied to filter samples. Pb, cadmium, Ni, and vanadium concentrations were measured using ATR-FTIR. One-way ANOVA, Fisher's protected LSD, and Dunnett-adjusted comparisons were used to evaluate the influence of geographic location against the field-reference site H1.
Results: H5 site showed highest levels of Ni and vanadium amounting to (0.987 and 1.910 mg/kg^{-1}), respectively. According to Dunnett-adjusted comparisons H1 site showed lowest levels of V compared to H3, H4, and H5 sites, however, a significant increase was observed in Ni content in H5 site. In addition, Pb and Cd levels reached (0.076 and 0.0533 mg/kg^{-1}), respectively, even though they did not differ much compared to H1 site. All recorded Pb levels were below the limit threshold (0.1 mg kg^{-1}) of honey. Petroleum residues and metal-binding processes were not independently confirmed by ATR-FTIR spectra, which were dominated by metal-binding mechanisms.
Novelty of the study: the assessment of (Pb, Ni, Cd, and V) in honey samples from hives near oil extraction infrastructural sites in Kirkuk, is one of the most significant colony-level assessments. This assessment incorporates the geographical and climate context with authorized elemental analysis.

**Corresponding Author:* *Ali M. Saadi, Email: ali.mohammed@ntu.edu.iq*

Conclusion: in preference to metal contamination, the element-specific pattern indicates a selective V-Ni accumulation in H5 site. Although source designation demands synchronized investigation of soil, dust, vegetation, pollen, and bee tissues, honey proved helpful as a screening matrix.

Keywords:
Apis mellifera; honey; food safety; nickel; vanadium; petroleum-related exposure; Kirkuk.

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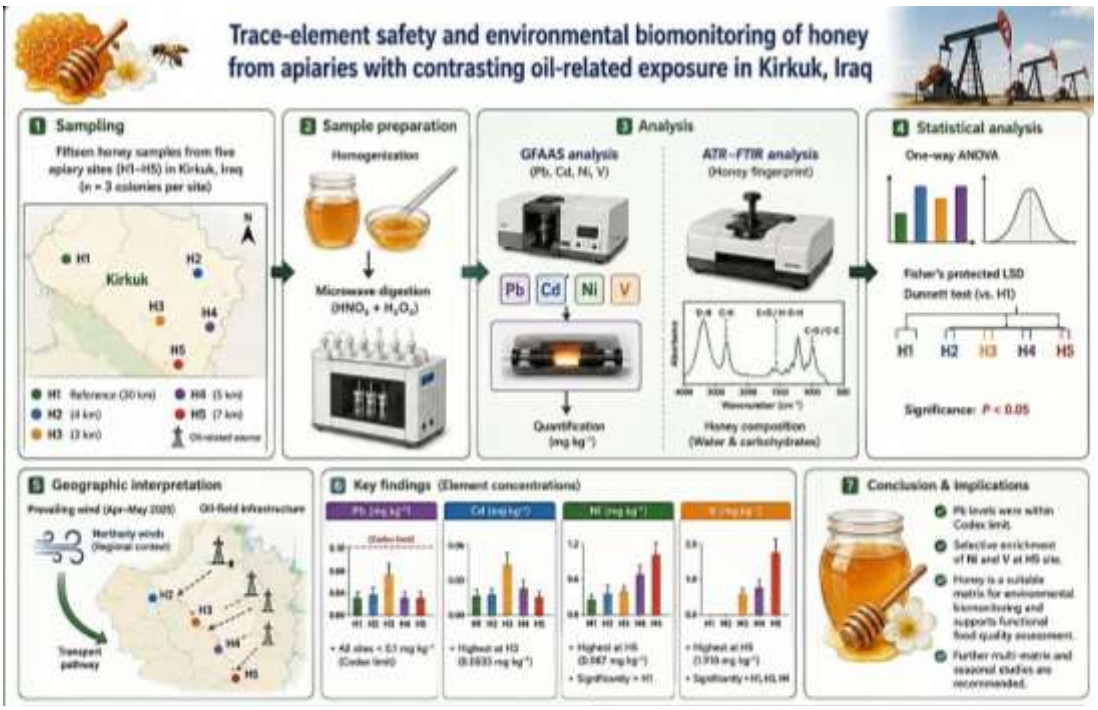

Graphical Abstract: Trace-Element Safety and Environmental Biomonitoring of Honey from Oil-Related Areas in Kirkuk, Iraq.

1. Introduction

Due to its abundance of vital ingredients for human health, honey is widely recognized as a natural product with great nutritional and functional value. Environmental factors influence the honey produced by honeybees due to their feeding habits and their interactions with flowers, water, dust, and airborne particles while foraging [1]. However, plant origin soil geochemistry, climate, and beekeeping protocols, in addition to beehive management influence metal concentrations in honey; as a result, geographical variations should be evaluated with caution [2].

Pb and Cd are considered as cumulative dietary pollutants. Ni is important for food safety due to the responses of people who are subtle, has developmental and reproductive outcomes, and subjected to prolonged chronic exposure to Ni [3]. Due to the lack of limits levels for Cd, Ni, or V, particularly in honey, Codex Alimentarius established a maximum Pb level of (0.1 mg kg^{-1}) [4]. No regulation limit related to honey regarding V levels exists, which is not considered as a nutrient, however, in areas where petroleum is produced, it provides environmental information.

Ni and V are elements naturally exists in crude oil, which can be enriched in fine particles formed during residual fuel incineration [5,6]. Kirkuk has a sizable processing and oilfield infrastructure. In addition, local inspections showed wind-related spatial variation in metals and petroleum-associated chemicals around oil-industrial sites [7]. Oil incineration and phosphate fertilizers have been recognized as potential source of V in Kirkuk topsoil [8]. Pb, Cd, Ni, and have also been shown to vary widely geographically in Iraqi honey studies [9,10].

Honey samples taken from hives, located in oil extraction sites in Kirkuk, were analyzed for levels of Pb, Cd, Ni, and V after comparing the pollution levels with reference sites. In particular, quantities of Ni and V were measured and compared with those related to inputs derived from petroleum. Pb concentrations were also compared to the Codex Alimentarius Code's allowable limits for honey, and the outcomes of ATR-FTIR assay were utilized to provide an additional description of the composition of honey.

2. Materials and Methods

2.1. Study area and honey sampling

Considering differing degrees of possible exposure to oil-related operations sites, five hives locations in Kirkuk Governorate were chosen. H1 site represents the control site, while H2-H5 sites show different distances from oil industrial centers. Three *Apis mellifera* colonies were sampled independently at each site between 10 and 20 June 2025 ($n = 3$ colony-level samples per site; total $N = 15$). Colony samples were kept separate throughout collection, storage, digestion, measurement, and statistical analysis. Each sample was stored frozen, equilibrated to room temperature, and homogenized before subsampling. Approximate distances to the nearest predefined oil-related source were estimated with Google Earth Pro (Table 1).

Table 1. Apiary-site characteristics and sampling design.

Site	Site description	Classification	Colonies, n	Sampling period	Approx. distance
H1	Remote agricultural area	Field reference	3	10–20 Jun 2025	30 km
H2	Near oil-related activity	Potentially exposed	3	10–20 Jun 2025	4 km
H3	Near oil-related activity	Potentially exposed	3	10–20 Jun 2025	3 km
H4	Arafah area	Potentially exposed	3	10–20 Jun 2025	5 km
H5	Sikaniyan area	Potentially exposed	3	10–20 Jun 2025	7 km

2.2. Digestion, GFAAS determination, and quality control

Approximately 0.5000 g of homogenized honey was treated with 7.0 mL HNO_3 (65%) and 1.0 mL H_2O_2 (30%), pre-reacted for 15 min, and microwave-digested at 200 °C. Digests were diluted to 25.0 mL. Pb, Cd, Ni, and V were measured with a PerkinElmer Analyst 800 GFAAS using Zeeman background correction and argon purge gas. Analytical wavelengths were 283.3, 228.8, 232.0, and 318.4 nm, respectively, in accordance with U.S. EPA Method 7010 [11]. Three successive instrumental readings were averaged for each colony sample; these were technical replicates and were not treated as additional biological observations.

Each batch included calibration and procedural blanks, calibration-verification standards, and pre-digestion matrix spikes. At least 5 non-zero references were included in the calibration. After calculating mass of samples, final volume, and dilution into consideration, the results were blank-corrected and represented as (mg kg^{-1}) honey. Values below the method detection limit were reported as <LOD rather than zero. The main performance characteristics are summarized in Table 2.

Table 2. GFAAS analytical and validation characteristics.

Element	λ (nm)	Range (µg L^{-1})	R^2	LOD/LOQ (mg kg^{-1})	Spike recovery (%)	RSD / CCV (%)
Pb	283.3	0.5–30	0.9992	0.006 / 0.020	92–106	3.5–7.5 / 96–104
Cd	228.8	0.05–5	0.9995	0.001 / 0.0035	94–108	2.8–7.0 / 95–105
Ni	232.0	1–50	0.9988	0.0185 / 0.060	90–110	4.0–9.0 / 95–106
V	318.4	1–80	0.9986	0.025 / 0.085	88–112	5.0–10.0 / 95–107

2.3. ATR-FTIR, spatial data, and statistics

ATR-FTIR spectra were obtained with a PerkinElmer Spectrum Frontier instrument fitted with a universal diamond ATR accessory. Spectra were recorded from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using four co-added scans. The technique was used descriptively to characterize water- and carbohydrate-dominated honey bands, not to quantify metals or prove petroleum residues [12,13].

Site mapping used approximate field locations. Regional wind information for late February to mid-June 2025 was obtained from Kirkuk meteorological station WMO 40621 and used as contextual evidence, not as a site-specific dispersion model. Each element was analyzed separately by one-way ANOVA, followed by Fisher's protected LSD for exploratory site comparisons and Dunnett-adjusted comparisons of H2–H5 against the a priori reference site H1 [14]. Significance was set at $P < 0.05$. Pb was compared with the Codex honey limit; Cd, Ni, and V were interpreted descriptively because honey-specific Codex limits were unavailable.

3. Results and Discussion

3.1. Element concentrations and comparisons with H1

As demonstrated in (table 3, figure 1) concentrations differed according to location and element. Ni and V levels were greater in H5 site, whereas Pb and Cd values peaked in H3 site. The divergence suggests that a single consistence source of contamination did not control the honey profile. Al Ni, Pb, Cd, and V showed significant general ANOVA results; however, the targeted Dunnett analysis revealed that only H5 site had considerably higher levels of Ni thanH1 site, while Pb and Cd levels did not significantly vary from their levels in reference site.

Table 3. Element concentrations and Dunnett comparisons with H1.

Site	Ni ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	V ($mg\ kg^{-1}$)
H1	0.387 ± 0.014^{c} Reference	0.053 ± 0.014^{ab} Reference	0.0316 ± 0.005^{ab} Reference	$<LOD^{c}$ Reference
H2	0.500 ± 0.023^{bc} P=0.846	0.063 ± 0.008^{a} P=0.843	0.0336 ± 0.003^{ab} P=0.999	$<LOD^{c}$ P=1.000
H3	0.320 ± 0.172^{c} P=0.971	0.076 ± 0.003^{a} P=0.279	0.0533 ± 0.017^{a} P=0.265	0.114 ± 0.006^{b} P=0.006
H4	0.800 ± 0.115^{ab} P=0.052	0.0327 ± 0.001^{b} P=0.348	0.0096 ± 0.001^{bc} P=0.255	0.134 ± 0.012^{b} P=0.002
H5	0.987 ± 0.092^{a} P=0.0066	0.028 ± 0.010^{b} P=0.213	0.0033 ± 0.010^{c} P=0.113	1.910 ± 0.041^{a} P<0.001
ANOVA P	**0.0041**	**0.0168**	**0.010**	**0.0001**

Values are mean ± SE (n = 3 colonies per site). Superscript letters denote Fisher's protected LSD groups. P values beneath H2–H5 are Dunnett-adjusted comparisons with H1.

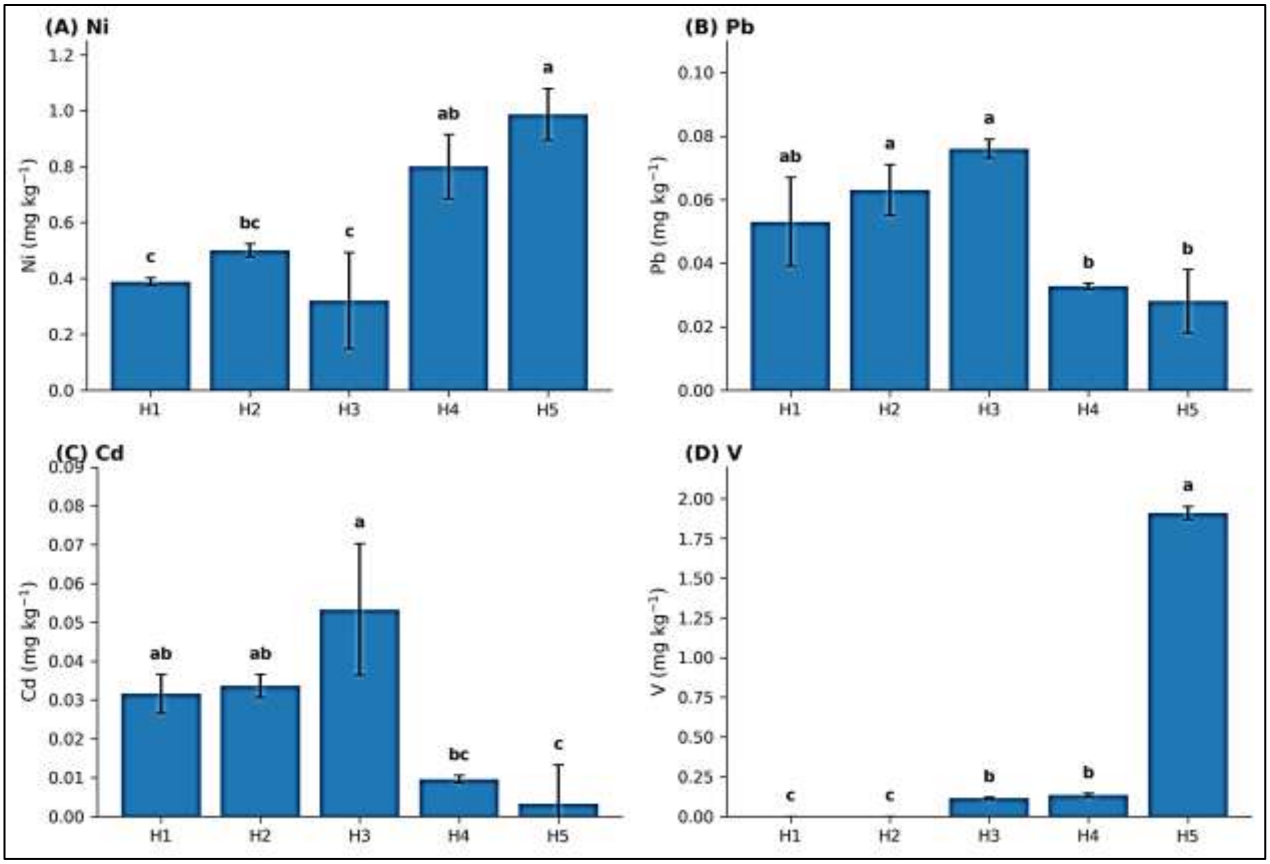

Figure 1. Trace-element concentrations across five apiary sites. Bars show mean ± SE; letters denote Fisher's protected LSD groups.

The only location to record a noticeably greater Ni level than site H1 was site H5, which had a high Ni level of (0.987 mg/kg^{-1}). At site H4, there was also a little rise in Ni level. The site-affected theory is supported by the fact that Ni level at H5 site was higher than the US permitted limit recording (0.516 mg/kg^{-1}) [15]. Several sources can be the reason of elevated levels of Ni, such as soil, oil extraction equipment, transportation, combustion particles, and petroleum wastes. As a result, it is wiser to to investigate it in combination with V rather that separately [5,6].

Pb and Cd levels varied according to the study outcomes, with site H3 demonstrating highest values, while comparing to site H1 (control), results showed no discernible difference. Nevertheless, every Pb measurement was below (0.1 mg/kg^{-1}) [4].

Cd levels at site H3 were notably higher than those found in low-background honey datasets, although they were still in line with findings from earlier researches [16]. These findings were also in line with the impacts of factors including transportation, local dust, beehives materials, and chemical structure of soil.

V concentrations were found to geographically vary with values below the limit of detection (LOD) at H1 and H2 sites, considerable fluctuation at H3 and H4 sites, and notably high at H5 site. However, in comparison to the H1 control site, V levels were noticeably greater at H3, H4, and H5 sites. Given that these elements are present in crude oil and residual fuel particles, the quantities of V and Ni at H5 sites could be linked to a potential particulate contribution from oil or combustion [5,6].

The recorded H5 V/Ni ratio was around (1.94), falling within the previously documented range of (1.4–2.1) for Kirkuk crude oil [17]. Due to the fact that elemental ratios can be altered by combustion, transportation, deposition, soil interactions, plant absorption, and bee-mediated transfer. This resemblance in encouraging but not conclusive.

3.2. Spatial and meteorological interpretation

Site specific variability was evident in V, with levels below (LOD) AT H2 site, and was intermediate at H3 and H4 sites, and high at H5 site. V concentrations were significantly higher at H3, H4, and H5 sites compared to H1 (control) site. These results are consistent with the simultaneous accumulation of V and Ni levels at H5 site, suggesting a particulate contribution of oil and combustion processes due to the presence of both elements in crude and residual fuel particles. It worth noting that H5 site was located at the greatest distance from the oil source relative to H2 and H4 sites. Contrary to expectations, the relationship between concentration and distance was not directly proportional, notwithstanding the high concentrations of V and Ni recorded. Furthermore, given that station WMO 40621 serves as a regional representative for Kirkuk rather than reflecting site-specific conditions, the prevailing northerly winds during April and May establish a transport pathway in southeasterly direction.

Credible sources include local soil and dust reservoirs: V levels in Kirkuk topsoil has been documented at (37–51 mg kg^{-1}), with phosphate fertilizers and oil combustion suggested as possible inputs [8]. There have also been reports of metal and petroleum-related substances dispersing due to wind near Kirkuk oil-industrial installations [7].

Potential exposure is not refuted by the absence of quantifiable V at H2 site. If a location is distant from an effective plume axis, if emissions are episodic, or if floral resources attract bees to a different forage sector, proximity by itself does not ensure deposition. Bee tissues were shown to be informative than honey for a number of elements in a research near Turkish thermal power plants [18]. Honey may also exhibit poorer reaction than bees or pollen for some metals. Thus, H5 site best described as a selective V-Ni hotspot when compared with oil-related particulate influence and/or locally enriched soil-dust, not as proof of transfer from a specific facility.

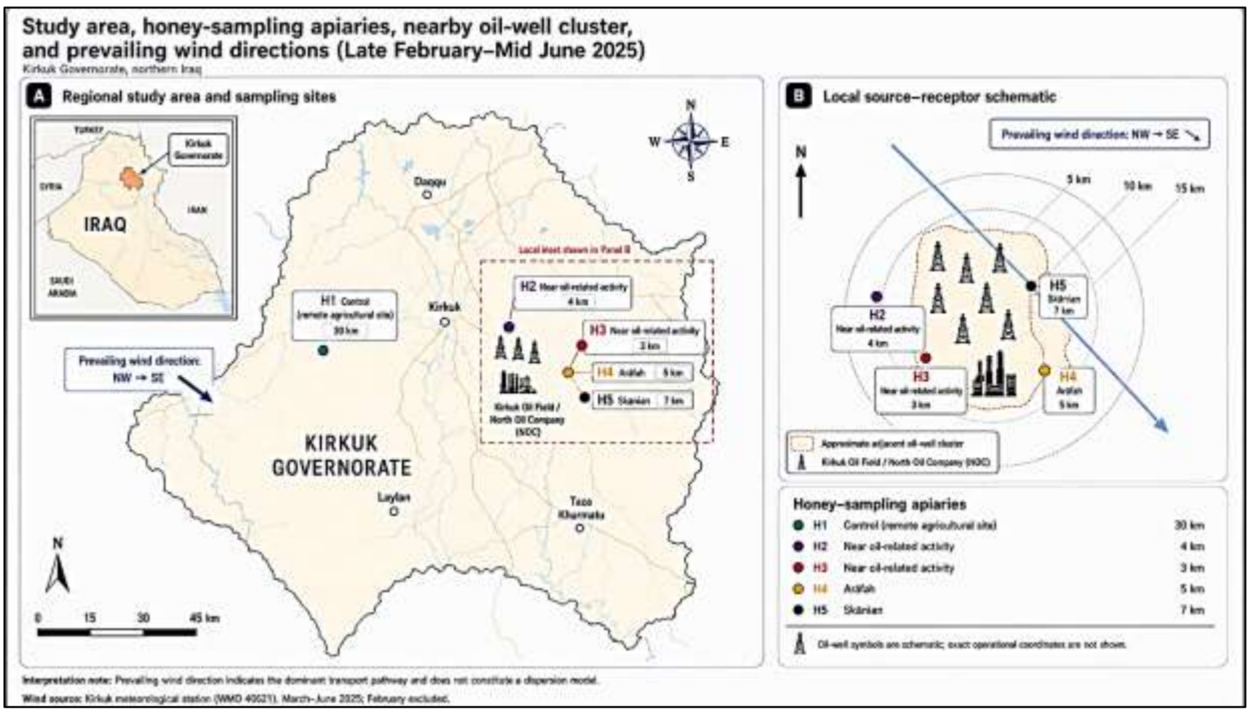

Figure 2. Sampling sites and prevailing wind direction in Kirkuk. Locations and oil-well symbols are approximate; the wind arrow provides regional context and is not a dispersion model.

3.3. ATR-FTIR spectral characteristics

All samples exhibited the characteristic O–H stretching region, aliphatic C-H bands, an absorption near 1640 cm^{-1}, and prominent carbohydrate-fingerprint signals indicative of honey (Figure 3; Table 4). The minor spectral features observed between 1995 and 2160 cm^{-1} remain unassigned, as they potentially arise from suggestions, atmospheric interference, baseline fluctuations, or instrumental noise. Consequently, ATR-FTIR analysis confirmed that the honey matrix is primarily composed of water and carbohydrates; however, it did not independently detect the presence of petroleum residues, silicates, metal oxides, specific metal-binding mechanisms [12,13].

Table 4. Main ATR-FTIR bands in honey samples.

Region (cm^{-1})	Samples	Tentative assignment	Interpretation
3661	H4	Free O–H	Possible moisture or hydrogen-bonding difference
3247–3276	H1–H5	O–H/N–H stretching	Water, sugars, organic acids, minor proteins
2972–2901	H1–H5	Aliphatic C–H stretching	Carbohydrate and organic-component bands
2160–1995	H1–H3, H5	Weak/unassigned	Not diagnostic without confirmatory analysis
1642–1638	H1–H5	H–O–H bending / C=O / N–H	Water with possible carbonyl/amide contribution
1251–1009	H1–H5	C–O and C–C stretching	Carbohydrate fingerprint region

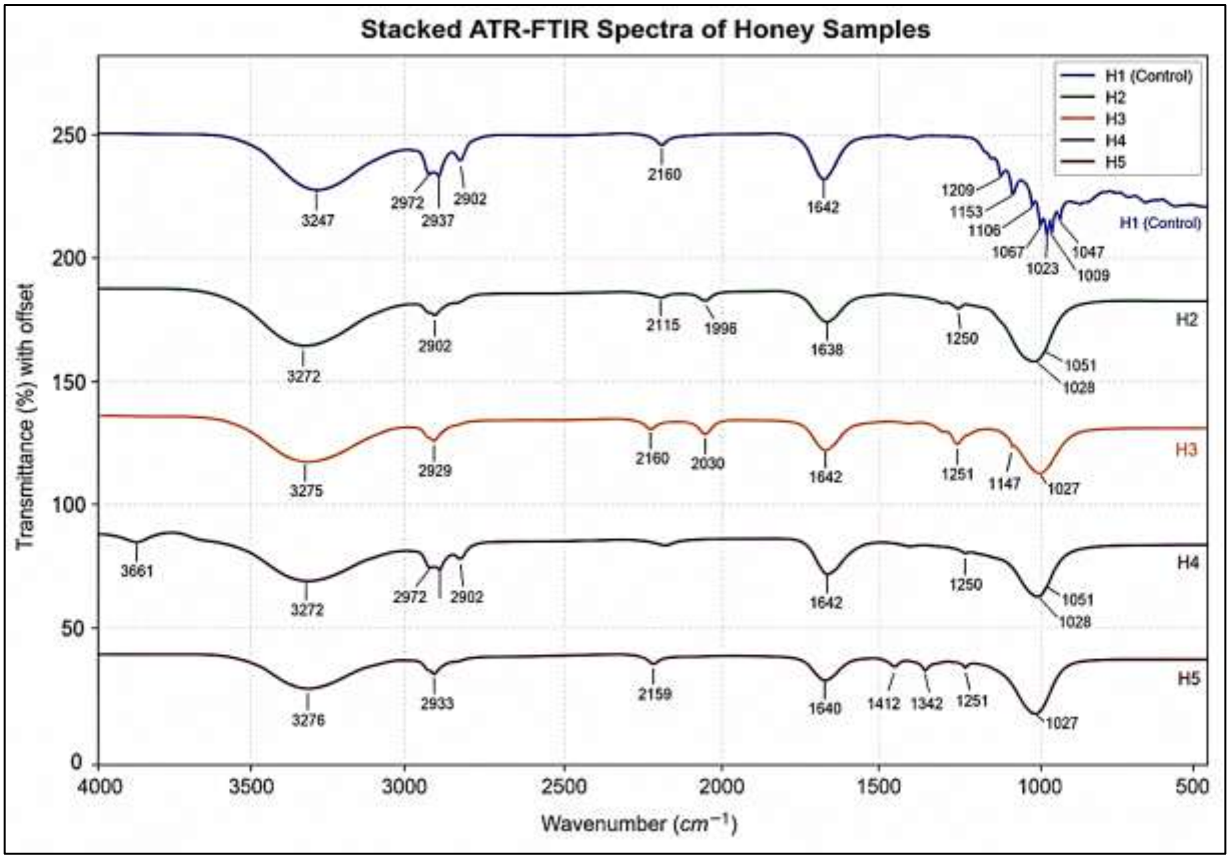

Figure 3. ATR-FTIR spectra of honey samples. Vertical offsets were applied for visual comparison; spectra are dominated by water and carbohydrate bands.

3.4. Functional-food relevance, limitations, and practical implications

Given that honey is regarded as avital meal for humans owing to its substantial nutritional advantages, it is crucial that honey quality inspection comprises confirming its authenticity and chemical composition as well as making sure it is free of any contamination. Pb levels in honey under investigation were found to be within allowable limits, indicating that it was Pb free. However, samples from H5 site demonstrated high levels of Ni and V, while samples from H3 site showed elevated levels of Cd, which provided important information on the degree of contamination. The lack in data regarding body weight, total dietary experience, and honey intake data, no consumer-risk assumption can be depicted. Honey, which is the sole environmental matrix exploited in the current study was restricted to individual production season, 3 colonies per location, and approximate source distances, therefor, according to this aim, source acknowledgment is still unclear.

Scientific innovation and practical implications:
Important information regarding Ni and V levels in honey from beehives localized in Kirkuk, a city with substantial oil production, was supplied by the current study. Compared to straightforward classifications between local and distant places, the data demonstrated the significance of each element's profile. This geographical and climatic study is important because it make it easier to check nutritional quality of honey and for environmental evaluation. Seasonal replication and parallel investigation of soil, settled dust forage plants, pollen, adult bees, and wax should be included in future research, and abnormal V and Ni values should be independently confirmed by ICP-MS or ICP-OES.

4. Conclusions

There were element-specific differences in Pb, Cd, Ni, and V levels between Kirkuk beehives. Whereas H3 site displayed the greatest levels of Pb and Cd, H5 site displayed the biggest contemporaneous V-Ni enrichment. Pb remained below the limit threshold specific to honey. The estimated honey matrix was validated by ATR-FTIR assay; however, pollution sources were absent. The findings validate honey as a screening bio-monitor and functional-food quality matrix; nevertheless, temporal replication and multi-matrix environmental monitoring are necessary for definite source identification.

List of abbreviations
ATR-FTIR, attenuated total reflectance Fourier-transform infrared spectroscopy; CCV, continuing calibration verification; GFAAS, graphite furnace atomic absorption spectrometry; LOD, limit of detection; LOQ, limit of quantification; NOC, North Oil Company; RSD, relative standard deviation; V/Ni, vanadium-to- Ni ratio; WMO, World Meteorological Organization.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Conceptualization, methodology, software, validation, formal analysis, investigation and resources, S. Dh. AND and z. A.; data curation, writing original draft preparation, writing—review and editing, visualization, supervision, M. Th. and S. Th.; funding acquisition, W.J. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate
Not applicable. The study analyzed honey products and did not involve human participants or experimental procedures on vertebrate animals.

Data availability
The data supporting the findings of this study are available from the corresponding author on reasonable request. High-resolution figures and calibration information should be provided as supplementary files during submission.

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