ANALYSIS OF HEAVY METALS PROFILE FROM BEE HONEY: A POTENTIAL MARKER FOR TOXIC METAL CONTAMINATION IN ECOSYSTEM

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Abstract:
An essential bee product with higher nutritional value is honey. Honey is also known to acquire trace metals that are both vital and poisonous. Furthermore, because bees collect nectar from practically all of the plants within a 100-kilometer radius, the quantity of hazardous metals in honey is a useful indication of toxic metal contamination in the ecosystem. Thus, in this study, the qualities of 40 honey samples with different origin standard physiological measures were used for the assessment. Like % moisture content, pH, specific gravity, refractive index, ˚Brix Index. Quantities measured included moisture content from 12.0% to 19.8 %; pH from 3.5 to 4.7; specific gravity from 1.35- 1.29 at 20℃; refractive index from 1.40-1.38 at 20℃; ˚Brix Index at 20℃ (Refractometer 0-90 range) from 84.66-76.53. The analysed samples demonstrated normal maturation, were free of unwanted fermentation, and agreed with standard values (Codex Alimentarius, 2000) and cited literature. After wet digestion, the amounts of trace heavy metals (Ag, Fe, Zn, and Ni) in Atomic absorption spectrometry readings were taken from honey samples. Honey samples were spiked with known concentrations of standard metals, and their recoveries were analysed to assess the method's precision. Honey samples were found to have minor metal concentrations between the of Ag~48.142 $mgkg^{-1}$, Fe~136.826 $mgkg^{-1}$, Zn~75.886 $mgkg^{-1}$ and Ni~47.294 $mgkg^{-1}$. The results found are consistent with a report in the literature and with codex limitations, and hence do not constitute a health hazard.

Keywords: Honey, Heavy metals, atomic absorption spectrometry, drench digestion

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Introduction:

Bees (*Apis mellifera*) collect nectar from flowers and turn it into honeydew, which they subsequently use to make honey (Codex Alimentarius Commission Standards, 2002). It's a complicated aqueous solution of sugars, and it's quite intense and deliquescent. Because plant species, temperature, weather, and the input of individual beekeepers all play a role in determining its content, honey can range widely. (Kurek-Górecka et al., 2020) It's important to note that honey has a wide variety of organic acids, sugars, hormones, enzymes, yeast, minerals, vitamins, and heavy metals (Pawel P et al., 2018). Honey may collect trace metals, including the poisonous metal nickel and the necessary elements zinc, iron, and silver, but only up to a specific concentration, which is safe for human health and development (Wolde Y et al., 2018). Nevertheless, excess ingestion of such components can still result in chronic toxicity (Ashenef, 2014). Because they have an apiary, hence access to an area of around 50 km2, this kind of bio-monitoring provides valuable insight about the bees' native ecosystem. (Cunningham, M. M., 2022). Since bees gather pollen and nectar from all elements of the environment—air, soil, and water—the heavy metal content in honey is indicative of total environmental levels (Parikh et al., 2021) honey's ability to serve as a biological indication of environmental pollution has been well acknowledged (Cunningham, M. M., 2022). Atomic absorption spectroscopy (FAAS, GFAAS) is only one of the various analytical techniques that has been used to study honey for its mineral and heavy metal content. (Bhartha et al., 2020) (Gajendra et al., 2016) the combination of mass spectrometry and inductively linked plasma-optical emission spectroscopy (Aghamirlou et al., 2015; Kılıç Altun et al., 2017). Recently, a comprehensive evaluation was conducted of the analytical techniques used and the values discovered for metal content of honey produced worldwide. (Solayman et al., 2016) India has a wide variety of flowering plants and excellent honey-making potential due to its varied topography, climate, and ecosystems. One of the first forms of farming in the United States was beekeeping. (Haldar et al., 2021). In terms of annual output, India placed seventh with 67,442 tonnes. Assuming a CAGR of 4.1% from 2020 to 2026, the value of the worldwide honey market is expected to increase from USD 6447.6 mn in 2020 to USD 8214.9 mn. The state of Uttar Pradesh produces the most honey (22,000 t), followed by the states of West Bengal (18,000 t) and Punjab (16,000 t). There was a 109.80% growth in India's honey exports from 28,378.42 MT in 2013-14 to 59536.75 MT in 2019-2020, with the total value of these shipments being to Rs. 6.3 billions, or 88.65 USD Millions (R Sharma, 2019). The goods acquired from this industry, however, are relatively modest in comparison to the country's potential. As a result, the researchers set out to determine how much of each trace metal was present in a variety of honey types. Beekeepers may use this knowledge to reduce the likelihood of contamination during honey processing, and consumers can use it to make educated decisions about the authenticity and safety of the honey they purchase.

2. Materials and methods

2.1. Sampling

Forty-one samples of honey from various locations were analysed in the research. Researchers gathered samples by randomly visiting honey shops and/or talking to local beekeepers in each municipality. After that, we combined and homogenised the samples before storing them in plastic tubs. The honey samples were shipped straight to the lab, where they will remain between 14 and 15 degrees Celsius until analysis can be performed.

Parameters	Ag (Silver)	Fe (Iron)	Zn (Zinc)	Ni (Nickel)
Wavelength (nm)	465.6	320.4	212.8	232.4
Spectral interval width (nm)	0.4	0.4	0.2	0.4
Supply current of discharge lamp (mA)	4.0	3.0	3.0	2.0
Background correction	no	no	no	no

Table 1: Absorption Quantities at the Atomic Level A) Wavelength (in nanometers) B) Spectral gap width (in nanometers) C) Background adjustment D) Discharge lamp supply current (mA)

2.2. Gadgetry

The device under discussion is an atomic absorption spectrophotometer that utilises both flame and graphite furnace systems. (**ICPOES Thermo brand Model 7000** series was used, the estimated reading time were 5 seconds, the RF power output were 1.2 kW, and the required stabilisation time were 15 seconds. The current viewing mode is set to Axial. The viewing height is not specified in millimetres. The recorded values for the plasma flow rate (U min) are 12, the auxiliary flow rate (U min) is 1, and the nebulizer

flow rate (U min) is 0.7. The correction limit were set at 0.99500. Metal analysis was conducted using equipment that was equipped with suitable hollow cathode lamps. The operational parameters of the selected components were established in accordance with the manufacturer's recommendations (Table 1). The flame was measured on a slit burner head measuring 10 cm in length. We also used a lamp and a flame with an air acetylene solution. Analytical balances (Mettler Toledo, Switzerland), Brix refractometer with ATC (SIMMANS INDIA PRIVATE LIMITED), pH meter (EUTECH INSTRUMENTS $_{PC}$510) were also used.

2.3 Preparation

Sample Preparation

2.3.1 Sample preparation can be done by either of the three methods based on requirement. For Microwave digestion, Weigh 0.5-1.0 gms of sample (based on sample nature) into digestion tube. Add 3 Ml Milli Q water. Add 8 mL nitric acid (HNO_3) and 1 mL hydrochloric acid (HCl) (if required, in case of Iron analysis). Leave the vessels for 30-45 minutes. Set the vessels in the rotor and run the digestion programme as per programming. After digestion make up the volume to 50 ml (Milli .Q Water). Run on Equipment for further analysis.

2.3.2 Indicator

Nitric acid (EMPARTA®), Hydrogen per Oxide (EMPLURA®), Sodium Hydroxide (EMPLURA®), all which are analytical grade from Merck Life Science Pvt. Limited Mumbai, and The 10^3 mg/L Ag, Fe, Ni, and Zn stock solutions are all authentic from reliable sources. (ISO 9001. ISO 17025. ISO 17034) were used.

2.4. Mechanism

2.4.1. Physicochemical analysis

After collecting honey from bees, the samples were optimised using AOAC,2000 procedures, for the purpose of measuring pH and determining the amount of moisture (free, lactone, and total). Readings were taken using a Brix refractometer at 20 degrees Celsius, and the percentage of moisture in the honey was calculated using the following table (AOAC, 2000). The pH was measured by dissolving 10 grammes of honey into 75 millilitres of decarbonated water in a 250 millilitre BOROSIL glass beaker and stirring the mixture with a magnetic stirrer for 30 minutes. After ensuring that all of the ingredients are mixed together evenly, the pH is determined by submerging a pH electrode in the solution. Lactic acid has a pH of 8.30. we first titrated the case study in contrast to a standardised 5×10^{-2} M NaOH until pH 8.50, then stopped adding NaOH at pH 8.50 (free acidity), added another 10 ml of NaOH, and back-titrated with HCl. The total acidity was calculated by summing the values for Liberated Lactonic acid (AOAC, 2000).

2.4.2. Examining of Metals

Following the guidelines provided by (Nega et al., 2020) we used this technique to break down honey samples. The procedure involved weighing and placing a 1 g sample of homogenised honey into a conical flask. After placing the sample in a flask, 8.0 ml of concentrated nitric acid and 4.0 ml of hydrogen peroxide were added. The sample combination was then dried out over a water bath at a high temperature for 4 hours. After the flask had cooled to room temperature, it was taken out of the water bath. After adding de-ionized water to the cooled sample to dissolve the dry bulk, Whatman no. 42 filter paper was used to filter the solution in a 10 ml volumetric flask. The solution was then diluted with deionized water to the desired concentration. Conc. Nitric acid and hydrogen peroxide are used together as a reagents to create the reagent blank, which was then processed in the same way as the sample. Next, an atomic absorption spectrophotometer was used to determine the optimal flask content based on the concentration of the desired trace metals (Ag, Fe, Ni, and Zn). Flame & graphite furnace system (**ICPOES Thermo brand Model 7000** series). As can be seen in Table 1. The recommended values for the instrument's parameters were followed. The instrument was adjusted to a known value using standard solutions of the metals under study. All steps were carried out three times for maximum accuracy.

3. Evaluation and Rationale

3.1. Physio parameters

Table 2. summarises the results of the study into the physio parameters of honey from various origins. The moisture level measured was in the range of 12.0%-19.8%. Honey's stability and resistance to spoiling due to yeast fermentation are directly related to its moisture content, making it a quality criteria. (Mertinotti et al., 2019). This quality also plays a significant role in establishing its storage stability (honey's shelf life) (Angioi et al., 2021). In accordance with honey samples evaluated showed no more than a 20% rate of adulteration, meeting the standards set by the European Community Directive (Codex Alimentarius Commission Standards, 2002). These results are consistent with ripe honey, suggesting

that the beekeepers extracted the honey at the ideal period. Variation in honeys' hydration levels can be attributed to many different things, such as their botanical roots, harvest seasons, and best times for extraction (Girma et al., 2017) All of the honey samples we've taken so far have acidic pH levels (3.50 - 4.75). It was discovered that honey made from Master Lichi Honey had the highest pH while honey collected from Kashmiri Acacia trees had the lowest. In accordance with the European Community Directive (3.5-5.5), all of the average pH levels fell within the acceptable range, with the exception of honey gathered from Kashmiri Acacia. Overall, the honey in Turkey had a mean pH of 3.96, which were on par with the export quality seen elsewhere in the world (Beram et al., 2021), Algeria, 3.47-4.44 (Otmani et al., 2019), Chili, 4.21 (Rodriguez et al., 2019), and Spain 3.58-4.76 (Rodriguez et al., 2019).

Sample Name	pH Value	% Moisture	Specific gravity(20°C)	°Brix at 20°C(Refractometer 0-90 range)	Refractive indexx at 20°C
Sarson Safeda	3.6	12	1.3525	84.66	1.4041
Mustard honey	4.10	12.2	1.351	84.45	1.4035
Kashmiri Acacia	3.50	12.4	1.3495	84.24	1.403
Kashmiri Multiflora	3.81	12.6	1.3481	84.03	1.4025
Master Lichi honey	4.75	12.8	1.3466	83.82	1.402
Neem Honey	3.6	13	1.3453	83.61	1.4015
Jamun honey	3.7	13.2	1.3438	83.39	1.401
Sunflower honey	4.5	13.4	1.3428	83.18	1.4005
Patanjali Honey	4.2	13.6	1.3409	82.97	1.3995
Concious Food Honey	3.8	14	1.3381	82.55	1.3985
Cucurmin Honey	3.65	14.2	1.3367	82.34	1.398
Hamdard Honey	3.81	14.4	1.3352	82.13	1.3975
Nature`s Nector Honey	4.21	14.6	1.3338	81.92	1.397
Wow Lichi Honey	4.23	14.8	1.3324	81.71	1.3965
Wow Ajwain Honey	3.91	15	1.331	81.56	1.396
Wow Forest Honey	3.82	15.2	1.3295	81.29	1.3955
Wow neem honey	3.23	15.4	1.3282	81.08	1.395
Wow Multifloral honey	3.55	15.6	1.3267	80.87	1.3945
Wow Berry honey	3.59	15.8	1.3254	80.66	1.394
Floral honey	4.52	16	1.3239	80.45	1.3935
Euclyptus Honey	3.57	16.2	1.3225	80.25	1.393
Anuras Multifloral honey	3.61	16.4	1.3212	80.04	1.3925
Saffola Honey	3.73	16.6	1.3197	79.83	1.392
Dabur Honey	3.55	16.8	1.3184	79.63	1.3915
Zandu Honey	3.56	17	1.3171	79.42	1.391
Dadev Honey	3.654	17.2	1.3156	79.21	1.3905
Hamdard Honey	3.612	17.4	1.3143	79.01	1.39
DiSano honey	4.36	17.8	1.3115	78.59	1.389
Apis Himalayan honey	4.31	18	13.101	78.39	1.3885
Organic India Multifloral honey	4.62	18.2	1.3087	78.18	1.388
24 Mantra Organic wild honey	4.26	18.4	1.3074	77.97	1.3875
Nature`s Nectar Honey	4.32	18.6	1.306	77.77	1.387
Bonphool Mangroove honey	4.61	18.8	1.3046	77.56	1.3865
Avni`s Tulsi Honey	4.16	19	1.3033	77.35	1.386
Avni` Rosewood Honey	4.27	19.2	1.302	77.15	1.3855
Avni`s Himalayan honey	3.95	19.4	1.3006	76.94	1.385
Avni`s Jamun Honey	3.72	19.6	1.2992	76.74	1.3845
Avni`s berry honey	3.54	19.8	1.2979	76.53	1.384
Codex[a]	3.5- 5.5	< 20			

Table 2: Physio Parameters. Codex Alimentarius Commission Standards (2002)[a] % Moistutre relationship with Specific Gravity and Refractive index and readings from Brix Refractometer (0-90 range)

3.2. Quantification of Metals

Honey samples were tainted with known concentrations of standard metals to test the method's efficacy in terms of recovery. In Table 3, we can see the metals' recovery rates, average results, limits of detection, and limits of quantitation. (Bergamo et al., 2019). These findings provide strong evidence for the reliability and validity of the techniques used to analyse honey samples. The consistency of the instrument's

readings (97.5%-102.1%) was demonstrated by the use of a standard adjustment for the interval of three samples. Absorbance was measured after triple digestion of six blank solutions (all treatment procedures for the analysis of sample were performed except for the addition of sample itself) of the limit of detection (LOD) and Limit of Quantification (LOQ) (Almeida et al., 2020) Metal concentrations in slivers of bee i.e honey were determined using an AAS equipment calibrated against an external standard. Metal content in honey samples ranged from silver (Ag) to zinc (Zn) to iron (Fe) to nickel (Ni). (**Table 4)** Each of the best honey samples tested positive for Ag, Fe, Zn, and Ni. The honey samples contained nectar from a wide variety of flowers, which explains the varying percentages of silver, iron, zinc, and nickel. (Generally speaking, soil and floral variances between foraging areas and the usage of galvanised containers are the two most common causes of metal contamination in honey (Flores et al., 2019). Maximum allowable levels of silver, iron, zinc, and nickel in honey according to the Codex Alimentarius Commission were not higher than 0.005mg/kg in any of the honey samples tested (Codex Alimentarius Commission Standards, 2002). It's possible that there's a connection between the different metal concentrations found in the honey samples and their respective locations (Lau et al., 2019), Recent changes in weather patterns and the presence of humans in these top honey-producing locations.

Sample Name	Elements	Avg, Result	% RSD	Recovery %	Unit	LOD	LOQ
1.Sarson Safeda	Ag	0.001	1.537	96.28	mg/kg	0.118	0.039
	Fe	91.541	5.31	90.57	mg/kg	0.202	0.665
	Zn	30.378	9.496	91.02	mg/kg	1.173	0.391
	Ni	3.81	2.557	86.97	mg/kg	0.086	0.028
2. Mustard Honey	Ag	3.067	0.711	95.28	mg/kg	0.128	0.033
	Fe	34.718	6.594	91.57	mg/kg	0.122	0.668
	Zn	12.226	4.081	92.02	mg/kg	1.163	0.395
	Ni	3.483	2.557	85.97	mg/kg	0.096	0.022
3.Kashmiri Acacia	Ag	2.786	2.1	94.28	mg/kg	0.108	0.038
	Fe	34.712	3.585	92.57	mg/kg	0.2	0.664
	Zn	12.262	2.675	91.02	mg/kg	1.163	0.397
	Ni	3.567	0.091	87.92	mg/kg	0.085	0.025
4.Kashmiri	Ag	0.001	1.537	91.02	mg/kg	0.119	0.034
	Fe	84.033	11.034	91.57	mg/kg	0.112	0.667
	Zn	31.535	6.445	85.97	mg/kg	1.174	0.393
	Ni	3.468	2.119	96.28	mg/kg	0.084	0.027
5.Master Lichi honey	Ag	3.011	2.119	91.57	mg/kg	0.119	0.035
	Fe	33.713	2.1	90.02	mg/kg	0.201	0.669
	Zn	12.097	2.676	87.97	mg/kg	1.172	0.397
	Ni	3.47	2.02	94.82	mg/kg	0.085	0.02
6.Neem Honey	Ag	2.919	1.537	91.75	mg/kg	0.118	0.035
	Fe	35.04	4.081	90.2	mg/kg	0.232	0.66
	Zn	12.522	3.854	85.79	mg/kg	1.154	0.399
	Ni	3.802	2.557	97.25	mg/kg	0.089	0.029
7.Jamun Honey	Ag	3.044	4.739	91.05	mg/kg	0.128	0.037
	Fe	36.378	1.544	92.29	mg/kg	0.212	0.667
	Zn	12.084	2.1	85.79	mg/kg	1.114	0.395
	Ni	3.702	3.567	86.28	mg/kg	0.189	0.021
8.Sunflower Honey	Ag	3.044	4.739	90.57	mg/kg	0.11	0.034
	Fe	36.378	1.544	91.86	mg/kg	0.2	0.669
	Zn	12.084	2.1	86.97	mg/kg	1.178	0.396
	Ni	3.702	3.567	85.18	mg/kg	0.089	0.023
9.Patanjali Honey	Ag	148.088	2.557	91.52	mg/kg	0.117	0.035
	Fe	33.529	2.1	90.18	mg/kg	0.205	0.666
	Zn	5.126	1.537	87.25	mg/kg	1.179	0.39
	Ni	2.96	1.544	96.22	mg/kg	0.08	0.029
10.Dadev Honey	Ag	2.537	4.315	92.36	mg/kg	0.11	0.038
	Fe	36.862	2.02	91.28	mg/kg	0.209	0.663
	Zn	12.093	3.854	85.38	mg/kg	1.176	0.397
	Ni	4.05	4.11	94.25	mg/kg	0.084	0.024
11.Concious food honey	Ag	0.001	1.537	94.26	mg/kg	0.115	0.037
	Fe	80.827	2.02	88.36	mg/kg	0.2011	0.665
	Zn	24.514	2.02	87.29	mg/kg	1.176	0.391
	Ni	2.96	1.544	93.12	mg/kg	0.086	0.026
12.Cucurmin honey	Ag	2.398	4.739	91.22	mg/kg	0.117	0.035

	Fe	38.207	3.093	90.13	mg/kg	0.204	0.662
	Zn	11.96	1.537	87.52	mg/kg	1.176	0.393
	Ni	3.471	2.119	96.42	mg/kg	0.089	0.02
13.Hamdard Honey	Ag	2.216	4.315	90.57	mg/kg	0.114	0.03
	Fe	37.406	3.659	92.25	mg/kg	0.205	0.668
	Zn	11.788	1.005	87.79	mg/kg	1.177	0.396
	Ni	3.791	2.557	96.28	mg/kg	0.088	0.023
14.Nature`s nectar	Ag	2.601	2.557	90.75	mg/kg	0.119	0.035
honey	Fe	38.818	4.602	91.21	mg/kg	0.206	0.668
	Zn	11.322	1.544	85.79	mg/kg	1.176	0.395
	Ni	4	3.124	95.82	mg/kg	0.085	0.02
15.Wow Lichi Honey	Ag	2.434	3.046	90.56	mg/kg	0.119	0.039
	Fe	37.983	2.712	91.26	mg/kg	0.212	0.668
	Zn	11.873	4.236	85.69	mg/kg	1.167	0.398
	Ni	3.88	2.712	96.75	mg/kg	0.078	0.023
16.Seasam Jandi Honey	Ag	0.001	1.537	91.28	mg/kg	0.123	0.037
	Fe	81.201	1.537	92.97	mg/kg	0.207	0.664
	Zn	27.559	2.557	85.89	mg/kg	1.179	0.399
	Ni	2.36	2.119	94.51	mg/kg	0.08	0.028
17.Wow Ajwain Honey	Ag	13.211	2.557	92.28	mg/kg	0.11	0.037
	Fe	243.24	2.544	90.57	mg/kg	0.207	0.664
	Zn	10.349	3.093	92.29	mg/kg	1.179	0.398
	Ni	2.858	1.537	85.59	mg/kg	0.085	0.027
18.Wow Forest Honey	Ag	2.786	2.557	94.28	mg/kg	0.113	0.032
	Fe	39.679	4.602	91.82	mg/kg	0.206	0.669
	Zn	11.972	2.544	93.03	mg/kg	1.178	0.394
	Ni	4.063	3.585	87.79	mg/kg	0.083	0.022
19.Wow neem honey	Ag	0.001	1.537	94.28	mg/kg	0.116	0.032
	Fe	78.819	2.544	91.57	mg/kg	0.207	0.669
	Zn	25.365	3.093	91.02	mg/kg	1.178	0.394
	Ni	2.715	2.02	86.97	mg/kg	0.084	0.022
20.Wow Multifloral honey	Ag	0.001	1.537	97.82	mg/kg	0.117	0.034
	Fe	84.576	3.262	90.56	mg/kg	0.209	0.668
	Zn	27.778	2.119	91.26	mg/kg	1.173	0.397
	Ni	3.11	5.239	87.47	mg/kg	0.085	0.026
21.Wow Berry honey	Ag	0.001	1.537	94.28	mg/kg	0.113	0.035
	Fe	81.889	12.034	90.75	mg/kg	0.202	0.6658
	Zn	26.865	1.724	92.28	mg/kg	1.173	0.397
	Ni	3.062	2.557	85.97	mg/kg	0.086	0.026
22.Floral honey	Ag	149.826	2.119	96.28	mg/kg	0.116	0.034
	Fe	35.548	3.854	91.57	mg/kg	0.208	0.669
	Zn	6.363	2.676	91.28	mg/kg	1.17	0.396
	Ni	3.173	1.544	96.28	mg/kg	0.084	0.029
23.Euclyptus Honey	Ag	0.001	1.537	90.57	mg/kg	0.117	0.032
	Fe	87.539	8.997	91.02	mg/kg	0.209	0.668
	Zn	30.754	6.958	86.97	mg/kg	1.17	0.394
	Ni	3.468	2.119	95.28	mg/kg	0.085	0.026
24.Anuras Multifloral honey	Ag	0.001	1.537	91.57	mg/kg	0.114	0.038
	Fe	91.541	5.31	92.02	mg/kg	0.207	0.667
	Zn	30.378	9.496	85.97	mg/kg	1.173	0.393
	Ni	3.81	2.557	94.28	mg/kg	0.082	0.026
25.Saffola Honey	Ag	2.216	4.315	92.57	mg/kg	0.119	0.03
	Fe	37.406	3.659	91.02	mg/kg	0.208	0.666
	Zn	11.788	1.005	87.92	mg/kg	1.179	0.392
	Ni	3.791	2.557	91.02	mg/kg	0.083	0.027
26.Dabur Honey	Ag	0.001	1.537	91.57	mg/kg	0.129	0.392
	Fe	84.576	3.262	85.97	mg/kg	0.218	0.022
	Zn	27.778	2.119	96.28	mg/kg	1.189	0.033
	Ni	3.11	5.239	91.57	mg/kg	0.093	0.668
27.DiSano honey	Ag	3.044	4.739	90.02	mg/kg	0.139	0.038
	Fe	36.378	1.544	87.97	mg/kg	0.228	0.664
	Zn	12.084	2.1	94.82	mg/kg	1.199	0.397
	Ni	3.702	3.567	91.75	mg/kg	0.193	0.025
28.Apis Himalayan honey	Ag	3.471	2.119	90.2	mg/kg	0.219	0.667
	Fe	2.216	4.315	85.79	mg/kg	0.108	0.393
	Zn	37.406	3.659	97.25	mg/kg	1.279	0.027

	Ni	11.788	1.005	91.05	mg/kg	0.383	0.035
29.Organic India Multifloral honey	Ag	2.786	2.1	92.29	mg/kg	0.129	0.66
	Fe	34.712	3.585	85.79	mg/kg	0.308	0.399
	Zn	12.262	2.675	86.28	mg/kg	1.159	0.029
	Ni	3.567	0.091	90.57	mg/kg	0.082	0.037
30.24 Mantra Organic wild honey	Ag	3.044	4.739	91.86	mg/kg	0.139	0.021
	Fe	36.378	1.544	86.97	mg/kg	0.208	0.034
	Zn	12.084	2.1	85.18	mg/kg	1.179	0.669
	Ni	3.702	3.567	91.52	mg/kg	0.383	0.396
31.Bonphool Mangroove honey	Ag	2.537	4.315	90.18	mg/kg	0.119	0.397
	Fe	36.862	2.02	87.25	mg/kg	0.251	0.02
	Zn	12.093	3.854	96.22	mg/kg	1.189	0.035
	Ni	4.05	4.11	91.56	mg/kg	0.083	0.66
32.Avni`s Tulsi Honey	Ag	3.81	2.557	91.57	mg/kg	0.119	0.034
	Fe	2.216	4.315	92.02	mg/kg	0.256	0.669
	Zn	37.406	3.659	85.97	mg/kg	1.176	0.396
	Ni	11.788	1.005	94.28	mg/kg	0.084	0.023
33.Avni` Rosewood Honey	Ag	84.576	3.262	92.57	mg/kg	0.12	0.029
	Fe	27.778	2.119	91.02	mg/kg	0.21	0.038
	Zn	3.11	5.239	87.92	mg/kg	1.169	0.663
	Ni	3.044	4.739	91.02	mg/kg	0.086	0.397
34.Avni`s Himalayan honey	Ag	26.865	1.724	91.57	mg/kg	0.111	0.037
	Fe	3.062	2.557	85.97	mg/kg	0.298	0.665
	Zn	149.826	2.119	96.28	mg/kg	1.169	0.391
	Ni	35.548	3.854	91.57	mg/kg	0.183	0.026
35.Avni`s Jamun Honey	Ag	37.406	3.659	90.02	mg/kg	0.319	0.393
	Fe	11.788	1.005	87.97	mg/kg	0.208	0.02
	Zn	84.576	3.262	94.82	mg/kg	1.179	0.03
	Ni	27.778	2.119	91.75	mg/kg	0.083	0.668
36.Avni`s berry honey	Ag	36.862	2.02	90.2	mg/kg	0.165	0.02
	Fe	12.093	3.854	85.79	mg/kg	0.207	0.03
	Zn	4.05	4.11	97.25	mg/kg	1.176	0.668
	Ni	3.81	2.557	91.05	mg/kg	0.085	0.396
37.Zandu Honey	Ag	3.702	3.567	92.29	mg/kg	0.11	0.023
	Fe	3.044	4.739	85.79	mg/kg	0.206	0.035
	Zn	36.378	1.544	86.28	mg/kg	1.171	0.668
	Ni	12.084	2.1	90.57	mg/kg	0.082	0.395
38.Dadev Honey	Ag	37.406	3.659	91.86	mg/kg	0.112	0.039
	Fe	11.788	1.005	86.97	mg/kg	0.205	0.668
	Zn	84.576	3.262	85.18	mg/kg	1.176	0.398
	Ni	27.778	2.119	91.52	mg/kg	0.086	0.023
39.Hitkary Honey	Ag	91.541	5.31	90.18	mg/kg	0.113	0.02
	Fe	30.378	9.496	87.25	mg/kg	0.201	0.03
	Zn	3.81	2.557	96.22	mg/kg	1.172	0.668
	Ni	3.067	0.711	91.56	mg/kg	0.089	0.396
40.Wild Forest Honey	Ag	24.514	2.02	90.15	mg/kg	0.118	0.02
	Fe	2.96	1.544	86.97	mg/kg	0.206	0.039
	Zn	2.398	4.739	97.82	mg/kg	1.174	0.668
	Ni	38.207	3.093	90.57	mg/kg	0.082	0.398

Table 3: Trace metal detection using ATOMIC ABSORPTION SPECTROPHOTOMETRY with %RSD, Recovery%, LOD(Limit Of Detection of trace metals) and LOQ (Limit of Quantification of trace metals)

Sample	Ag mg/kg	Fe mg/kg	ZnO mg/kg	NiO mg/kg
Sarson Safeda	0.003 ±0.02	0.001 ±0.02	0.004 ±0.02	0.001 ±0.02
Mustard	0.004 ±0.01	0.003 ±0.01	0.003 ±0.01	0.003 ±0.01
Kashmiri Acacia	0.006 ±0.01	0.002 ±0.01	0.002 ±0.01	0.005 ±0.01
Kashmiri Multiflora	0.003 ±0.02	0.001 ±0.02	0.004 ±0.02	0.001 ±0.02
Master Lichi Honey	0.004 ±0.01	0.003 ±0.01	0.003 ±0.01	0.003 ±0.01
Neem Honey	0.001 ±0.02	0.002 ±0.02	0.002 ±0.02	0.002 ±0.02
Jamun Honey	0.002 ±0.03	0.004 ±0.03	0.002 ±0.03	0.004 ±0.03
Sunflower	0.003 ±0.02	0.003 ±0.02	0.006 ±0.02	0.006 ±0.02
Patanjali Honey	0.005 ±0.01	0.006 ±0.01	0.002 ±0.01	0.002 ±0.01
Dadev Honey	0.002 ±0.02	0.001 ±0.02	0.001 ±0.02	0.003 ±0.02
Concious Honey	0.004 ±0.01	0.003 ±0.01	0.001 ±0.01	0.001 ±0.01
Cucurmin Honey	0.003 ±0.03	0.002 ±0.03	0.003 ±0.03	0.002 ±0.03
Hamdard honey	0.002 ±0.01	0.006 ±0.01	0.002 ±0.01	0.003 ±0.01

Nature`s Nector Honey	0.001	±0.02	0.002	±0.02	0.002	±0.02	0.001 ±0.02
Wow Lichi Honey	0.004	±0.01	0.003	±0.01	0.001	±0.01	0.005 ±0.01
Wow Ajwain Honey	0.003	±0.01	0.001	±0.01	0.002	±0.01	0.003 ±0.01
Wow Forest Honey	0.004	±0.01	0.003	±0.01	0.003	±0.01	0.002 ±0.01
Wow neem honey	0.006	±0.02	0.005	±0.02	0.004	±0.02	0.001 ±0.02
Wow Multifloral honey	0.001	±0.03	0.002	±0.03	0.002	±0.03	0.002 ±0.03
Wow Berry honey	0.002	±0.02	0.001	±0.02	0.006	±0.02	0.001 ±0.02
Floral honey	0.003	±0.02	0.003	±0.02	0.001	±0.02	0.005 ±0.02
Euclyptus Honey	0.005	±0.02	0.004	±0.02	0.004	±0.02	0.006 ±0.02
Anuras Multifloral honey	0.003	±0.01	0.002	±0.01	0.005	±0.01	0.002 ±0.01
Saffola Honey	0.002	±0.01	0.001	±0.01	0.003	±0.01	0.001 ±0.01
Dabur Honey	0.001	±0.01	0.003	±0.01	0.002	±0.01	0.003 ±0.01
Zandu Honey	0.006	±0.02	0.005	±0.02	0.003	±0.02	0.005 ±0.02
Dadev Honey	0.003	±0.03	0.001	±0.03	0.001	±0.03	0.004 ±0.03
Hamdard Honey	0.001	±0.02	0.003	±0.02	0.002	±0.02	0.003 ±0.02
Saffola Honey	0.006	±0.04	0.001	±0.04	0.003	±0.04	0.001 ±0.04
DiSano honey	0.003	±0.03	0.002	±0.03	0.002	±0.03	0.002 ±0.03
Apis Himalayan honey	0.002	±0.02	0.001	±0.02	0.001	±0.02	0.001 ±0.02
Organic India Multifloral honey	0.004	±0.02	0.003	±0.02	0.003	±0.02	0.003 ±0.02
24 Mantra Organic wild honey	0.001	±0.02	0.002	±0.02	0.002	±0.02	0.002 ±0.02
Nature`s Nectar Honey	0.003	±0.01	0.003	±0.01	0.001	±0.01	0.004 ±0.01
Bonphool Mangroove honey	0.001	±0.01	0.001	±0.01	0.003	±0.01	0.002 ±0.01
Avni`s Tulsi Honey	0.003	±0.01	0.003	±0.01	0.002	±0.01	0.005 ±0.01
Avni` Rosewood Honey	0.006	±0.03	0.004	±0.03	0.001	±0.03	0.003 ±0.03
Avni`s Himalayan honey	0.001	±0.03	0.001	±0.03	0.003	±0.03	0.002 ±0.03
Avni`s Jamun Honey	0.004	±0.03	0.005	±0.03	0.006	±0.03	0.002 ±0.03
Avni`s berry honey	0.003	±0.02	0.002	±0.02	0.002	±0.02	0.004 ±0.02

Table 4: Concentration of studied heavy metals mg/kg after wet digestion of honey samples

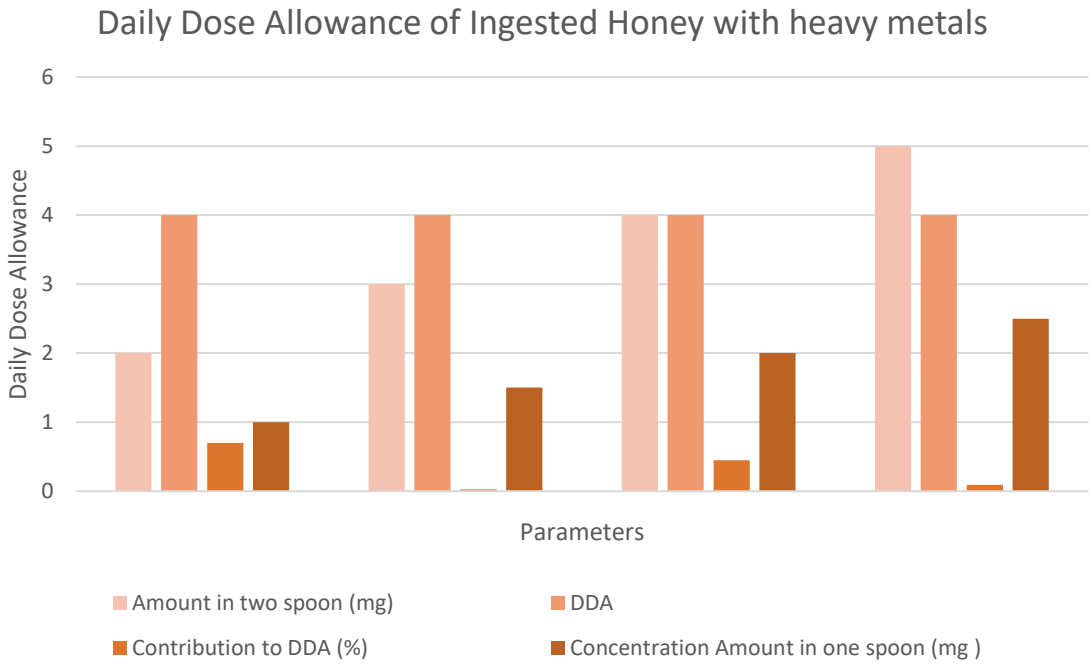
3.3. Assessment of the Nutrient Content of Honey Samples

Weights of both 1 and 2 teaspoons of honey from each kind were taken to determine their nutrient density. The honey samples were analysed to determine the concentration of the components therein, and the contents of one and two spoons of honey were compared to the respective D.D.A. (Bella et al., 2022). This analysis makes an effort to contrast four widely researched metals (Ag, Fe, Zn, and Ni). Honey's impact on recommended daily allowance, as summarised by two teaspoons, is seen in Table 5. It's hard to draw parallels between the harmful effect of heavy metals investigated and the continuous and rapid absorption of honey because of the lack of a declared daily consumption of honey. Honey provides anything from 0.025 percent to 6.38 percent of the daily recommended intake (D.R.I.) when consumed in the recommended amount (2 tablespoons).

Sample	Concentration Amount in one spoon (mg)	Amount in two spoon (mg)	DDA	Contribution to DDA (%)
Sarson Safeda	01	02	04	0.7
Mustard	1.5	03	04	0.025
Kashmiri Acacia	02	04	04	0.45
Kashmiri Multiflora	2.5	05	04	0.09
Master Lichi Honey	3	06	04	0.28
Neem Honey	3.5	07	04	0.46
Jamun Honey	4	08	04	0.39
Sunflower	4.5	09	04	0.28
Patanjali Honey	5	10	04	0.3
Dadev Honey	5.5	11	04	0.64
Concious Honey	6	12	04	0.56
Cucurmin Honey	6.5	13	04	0.44
Hamdard honey	7	14	04	0.21
Nature`s Nector Honey	1.1	2.2	02	0.25
Wow Lichi Honey	2.1	4.2	02	0.35
Wow Ajwain Honey	3.1	6.2	02	0.14
Wow Forest Honey	4.1	8.2	02	0.64
Wow neem honey	5.1	10.2	02	0.23
Wow Multifloral honey	6.1	12.2	02	0.89
Wow Berry honey	7.1	14.2	02	074
Floral honey	8.1	16.2	02	0.14
Euclyptus Honey	9.1	18.2	02	0.69

Anuras Multifloral honey	10.1	20.2	02	0.22
Saffola Honey	11.1	22.2	02	0.29
Dabur Honey	12.1	24.2	02	0.33
Zandu Honey	13.1	26.2	02	0.31
Dadev Honey	14.1	28.2	02	0.37
Hamdard Honey	15.1	30.2	02	0.62
Saffola Honey	16.1	32.2	02	0.55
DiSano honey	17.1	34.2	02	0.41
Apis Himalayan honey	01	02	01	0.32
Organic India Multifloral honey	02	04	01	0.21
24 Mantra Organic wild honey	03	06	01	0.61
Nature`s Nectar Honey	04	08	01	0.121
Bonphool Mangroove honey	05	10	01	0.332
Avni`s Tulsi Honey	06	12	01	0.45
Avni` Rosewood Honey	07	14	01	0.51
Avni`s Himalayan honey	08	16	01	0.14
Avni`s Jamun Honey	09	18	01	0.47
Avni`s berry honey	10	20	01	0.63

Table 5: One and two teaspoons of honey have different amounts of heavy metals Concentration w.r.t contribution to D.D.A %.



4. Conclusion

The physicochemical characteristics, including moisture content, pH, %moisture, specific gravity, refractive index, and Brix refractometer readings, of honey produced by the bees from various flora and fauna gathered from various geographical places were evaluated. Concentrations of other trace metals (Ag, Fe, Zn, and Ni) were also calculated. The values found are consistent with those suggested by the Codex. Samples from different locations were found to have vastly variable levels of the researched physicochemical characteristics and heavy metals. Geographical considerations, the proximity of bee foraging areas to roadsides, soil type, and anthropogenic sources all likely play significant roles in the observed range of values.

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